UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
GRIGORE T. POPA IAȘI

Optimizing Deaf Children´s Journey to the Hearing World

- HABILITATION THESIS -

LUMINIȚA MIHAELA RĂDULESCU MD, PhD

Iași – 2019

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THESIS SUMMARY

My scientific, professional and academic activities were focused on several directions like skin cancer of the face and of the external ear, sleep apnea in children, pathology of the middle and inner ear (hearing loss and vertigo), but the most important one is the diagnosis and treatment of hearing loss in children.

The thesis presents the main results of my post-doctoral scientific work mainly on rehabilitation of congenital deaf children. Hearing loss represents a worldwide problem affecting the health of over 466 million people. The main objective of my scientific work was to optimize the rehabilitation of congenital deaf children, the final aim being to introduce them into the hearing world - culminating in the inclusion of these children in the mainstream school. It was a complex task that included some well defined etapes, each and all of them irreplacebal: early detection of congenital hearing loss, early diagnosis, early cochlear implantation, personalized fitting and speech rehabilitation.

The thesis begins with the summary in English and Romanian followed by a brief presentation of the accomplishments on professional, academic and scientific level. The elements of national and international recognition are mentioned as well.

The thesis itself is structured in three main sections.

The first section is the largest part and is dedicated to scientific achievements relating to our results regarding the optimizing methods of detection, diagnosis and treatment of hearing-impaired children with a special emphasis on profound congenital neurosensorial hearing loss.

The development of speech in child is conditioned by the presence of hearing at birth. Early detection and diagnosis up to 3 months of age, followed by early intervention, by the age of 6 months and cochlear implantation until 12 months of age whenever indicated, are the key steps for an optimum rehabilitation. Through the sustained efforts of our team the screening program for neonatal hearing loss was started in Iasi. The results of the first 6 years of screening are presented. Results of a second pilot study of hearing loss in the primary school child are also presented. These studies have been a reference for the introduction of neonatal national screening in Romania beginning with this year. We continued to develop the program by conducting genetic screening of connexin 26 mutations in Iasi inside of an international research project won by competition.

We have developed a battery of speech audiometry tests in Romanian - the first of its kind in our country dedicated to primary school children.

Another study, conducted in collaboration with the University of Freiburg, refers to optimization of the etiological diagnosis of deafness. The study included over 200 children cochlear implanted in our clinic. Genetic analysis of mutations in the GJB2 gene found mutations of Connexin 26 in more than 48% of children. In a second phase, some rare mutations in GRXCR1, ESPRB, TMIE, GIPC and LHFPL5 genes were searched.

The surgical technique of cochlear implantation was personalized. The size of the cochlea was measured in order to implant the most suitable electrode; surgical intervention was adapted regarding the size of the incision and the insertion method of the electrode. Also, some methods for individualized fitting were proposed.

In the second section I present my future projects. Among them are the following:

- Optimization of the genetic tests for the mutations responsible for hearing loss.
- Identification of factors responsible for speech rehabilitation of the implanted congenital deaf children.
- Evaluating the outcome of simultaneous implanted vs sequential implanted children and also to establish the optimum interval between the two surgical cochlear implantations in cases of sequential.
- The presence of the biofilm in the healthy middle ear and at the level of the cochlear implants in the animal model.
- Development of new drug-loaded nanocarriers designed to treat some inner ear disorders.

The third section includes the list of references.

REZUMATUL TEZEI

Activitatea mea științifică, profesională și academică s-a concentrat pe mai multe direcții cum ar fi neoplazia cutanată a feței și urechii externe, apneea de somn la copii, patologia urechii medii și interne (hipoacuzia și vertijul), dar cea mai importantă direcție de cercetare a fost cea legată de diagnosticul și tratamentul hipoacuziei la copii.

Teza de față prezintă principalele rezultate ale activității mele științifice post-doctorale (din 2003) privind reabilitarea copiilor surzi congenitali. Obiectivul principal a fost de a optimiza rezultatele reabilitării copiilor surzi congenital și introducerea lor în lumea sunetelor - culminând cu includerea în școala de masă. Această activitate complexă a cuprins etape bine definite, fiecare etapă fiind decisivă în recuperarea copilului: detectarea precoce a pierderii auzului, diagnosticarea precoce, tratamentul precoce personalizat și reabilitarea vocală.

Teza începe cu rezumatul în limbile engleză și română, urmată de o scurtă prezentare a realizărilor la nivel profesional, academic și științific. Elementele de recunoaștere națională și internațională sunt de asemenea menționate.

Teza în sine este structurată în trei secțiuni principale.

Prima secțiune este cea mai vastă fiind dedicată prezentării rezultatelor activității de cercetare în domeniul optimizării metodelor de diagnostic și tratament al copiilor cu hipoacuzie cu un accent deosebit pe hipoacuzia neurosenzoriala congenitală profundă.

Dezvoltarea vorbirii la copilul cu hipoacuzie profundă congenitală este condiționată de prezența auzului la naștere. Pentru a optimiza rezultatele reabilitării este necesar ca acești copii să fie depistați precoce și diagnosticați până la vârsta de 3 luni, reabilitarea să înceapă până la vârsta de 6 luni iar în cazul în care există indicația de implantare cohleară aceasta să se realizeze până la vârsta de maxim 12 luni.

Prin eforturile susținute ale echipei noastre a fost demarat programul de screening al hipoacuziei la nou născut în maternitățile din Iași și din acest an și în țară. În teză sunt prezentate rezultatele primilor 6 ani de screening precum și rezultatele unui studiu pilot de depistare a hipoacuziei la copilul din școala primară. Aceste studii au fost de referință pentru introducerea screening ului național neonatal in Romania începând din acest an (2019). Am continuat să dezvoltăm domeniul prin efectuarea în Iași a screening-ului genetic al mutațiilor de la nivelul conexinei 26 în cadrul unui grant internațional de cercetare câștigat prin competiție.

O altă preocupare a fost aceea de a optimiza testele audiologice pentru copiii de vârstă școlară. În acest scop am elaborat o baterie de teste de audiometrie vocala în limba romana – prima și unica de acest fel din țara noastră.

Un alt studiu, efectuat în colaborare cu Universitatea din Freiburg, a urmărit optimizarea diagnosticului etiologic al hipoacuziei. Studiul a inclus peste 200 de copii implantați cohlear la noi în Clinică. Analiza genei GJB2 a relevat mutații ale Conexinei 26 la peste 48% din copii. Într-o a doua etapă au fost evaluate mutațiile mai rare de la nivelul genelor GRXCR1, ESPRB, TMIE, GIPC si LHFPL5.

Am urmărit personalizarea tratamentului copilului cu surditate. Pentru optimizarea implantării cohleare am realizat măsurători ale cochleei pentru a alege cel mai potrivit electrod, am adaptat intervenția chirurgicală în ceea ce privește mărimea inciziei și modul de inserție al electrodului. De asemenea, am propus o nouă metodă de personalizare a reglajului electrozilor.

In cea de a doua secțiune sunt prezentate proiectele de viitor dintre care amintim:

- Identificarea factorilor responsabili de rezultatele reabilitării prin implant cohlear
- Identificarea intervalului maxim între intervenții în implantarea secvențială astfel încât rezultatele să fie comparabile cu cele obținute în implantarea simultană
- Studiul biofilmului de la nivelul urechii medii și de la nivelul implantului cochlear
- Participarea la crearea de nanoparticule care să transporte drogurile către urechea internă.

Ultima secțiune cuprinde reperele bibliografice.

CAREER DEVELOPMENT AS A CONTINUUM

- professional, academic and scientific contributions –

Career overview - My Curriculum Vitae covers all the information concerning my career development. I graduated the University of Medicine and Pharmacy "Gr. T. Popa " Faculty of Medicine in Iaşi in 1989. After a short period of internship, I became a secondary doctor (resident doctor these years) in Otolaryngology through national residency exam in 1992. In 1995 I took the position of assistant professor, then lecturer, followed by associate professor in 2014.

I have been working as a teaching staff in UMF Grigore T. Popa Iasi since October 1995 (over 24 years of activity). I received the title of attending physician (medic primar) in 1999.

In 2003 I was certified doctor in medicine by Order MEC nr.5663.

I participated in 6 research grants won by competition:

- 1 of them won by international competition as project coordinator and has to be noted that was classified on the first place.
- 4 of them won by national competition:
- 1 won by intern competition – UMF Iași – as team member.

I have publishes over 60 papers *in extenso* in BDI since PhD thesis, 20 of them published in ISI journals.

Six books written in the field of ORL.

Over 160 citations (h-index 6) in Google Scholar / Over 100 citations (h-index 6) in ISI Thomson

I am the Head of the Cochlear Implant Compartment since 2007, and Head of ORL Clinic since 2008 and Residents coordinator.

Member of medical Council in Rehabilitation Hospital

Member of the Ethic Commission of the Rehabilitation Hospital

Appointed member in PhD Commissions

Regional Coordinator of the National Sub-Program within the National Health Program of Hearing Rehabilitation through Cochlear Implant and other implantable prosthesis since 2009 – prezent.

President of European Symposium of Paediatric Cochlear Implantation - Bucharest, 2019.

Vice-president of National ORL Congress – Iași, 2010

I was part of the surgical team (Prof. D. Martu and I) that performed the first ever Cochlear implantation in Romania in March 2000.

All this time I continued to work as a clinician and a surgeon in the ORL Clinic from Rehabilitation Hospital Iași. This Clinic covers also pediatric ORL (there is an ORL Paediatric Compartment) as well as hearing rehabilitation, in both children and adults, using implantable devices in the Cochlear Implant Compartment (where all types of hearing implantable devices available on Romanian market are implanted, namely: cochlear implants from all 4 Companies, Baha, Vibrant Sound Bridge and Bone Bridge – the only Clinic in Romania that covers all the area of implantable hearing aids).

Appointed member of the editorial board of:

- Journal of Dentistry and ORL.ro Magazine – both indexed BDI
- Journal of the Romanian Society of Otolaryngology and Cervico-Facial Surgery

Two prizes for research results (UEFISCU) – articles in 2013, 2014.

Invited speaker in more than 15 international leading conferences.

Visiting Professor of the State University of Medicine and Pharmacy ”Nicolae Testemițanu” of the Republic of Moldova.

Academic activity - My teaching activities (courses and workshops) addressed to:

- Students to the following faculties: Medicine (Romanian and English languages), Dentistry and Bioengineering
- Residents: ENT, Occupational Medicine and Orthodontics, and also
- Specialists: ORL, General Practitioner, Neurology etc.

with the aim of developing skills in line with current international professional standards, of introduction of new courses on previously unrelated directions aligned with the scientific progress in the field of activity.

Faculty of Dentistry - An important achievement was the introduction of an optional course: ENT Emergency, for the Faculty of Dentistry, starting with the academic year 2006-2007. Another direction I initiated was inclusion of a new theme in the mandatory course: Obstructive Sleep Apnea - relevant pathology for the Faculty of Dentistry graduates.

Faculty of Bioengineering - During 2002-2005 I organized the Ear Implants course at the Faculty of Bioengineering - Section of Prostheses Technology - the 6th year of study. This constituted the basis of the opening of a research line of the implant-neurosensory interface that linger until now a day, and which involves me to a significant extent in the interactions with PhD students.

College of Audiology - During 2002-2004, along with my colleagues I developed, on the basis of both our own experience and on an updated international bibliography, part of the analytical programs for the courses and practical works of the Audiology College (for Objective Audiology, Clinical Audiology, ORL Semiology, Clinical Vestibulogy).

Master-degree in Speech Therapy - I gave lectures to the students enrolled in Master-degree of Speech Therapy in order to enhance the knowledge of auditory pathology and phonation thus, to create the basis for a qualified trained professional. Through the clinical cases presented, I highlighted the importance of the ORL examination of the patient with dysphonia or with language development disorder.

I coordinate over 20 continuous medical education programs.

I was lecture in over 30 continuous medical education programs.

The teaching activity that I have sustained over the 24-year period addressed to the students / nurses / master degree students / specialists has registered a continuous development (both in terms of personal perception and of the effectiveness and extension of the training). It was supported by a continuous effort of bibliographic documentation; it was validated in clinical practice and was engaged in research areas relevant for the teaching process.

Over the years, I introduced computer-assisted presentation of lectures focusing on graphics, photos and movies to reflect the teaching problem more clearly. Courses have been explicit elaborated providing judicially selected and up-to-date medical information. Their interactive character was highly appreciated by the students.

I have developed an attractive, interactive software showing the surgical techniques for reconstruction of the skin defects of the nose ("Decision Making Model for the Reconstruction of the Nasal Skin Defects after Carcinoma Excision"). This learning software addresses to ORL residents doctors.

I am the main author or co-author of six books in the field of ORL dedicated to the medicine students, nurses, ORL residents and ORL specialists.

I have coordinated the Scientific Section of Otology and Neurotology of the Society of Physicians and Naturalists Iași where case reports, scientific discussions or surgical techniques presentations were made.

I have supervised 30 dissertations – but more than, how to write, I thought that it was important to teach them how to conduct a research and then how to write the paper.

I have attended the admission commission in university in the first years. In the last 5 years I attended the residency exam.

Since 2008 I was appointed each year member of the commission for ORL specialist and consultant degree exams. In 2016 I was appointed president of the commission for specialist degree exam.

I have also been a member of many promotion and doctoral commissions in our own university and in other universities.

I have coordinated the practical training of residents in different specialties (ORL, Orthodontics, Neurosurgery, Plastic surgery, Occupational medicine, Allergology) and also, I gave them lectures.

Research activity - The research activity continued after the bachelor's thesis and was amplified by rallying to European prestigious research groups (Freiburg, Tübingen, Lyon) and highlighted in some publications.

From the perspective of the coordination of the above-mentioned scientific circle, I achieved the continuous updating of the knowledge in the area of research in the field of competence, which allowed the research projects to be approached on a realistic basis, some already performed, others in progress or in the process of obtaining the financing.

At present, much of the research effort is invested in a border area, namely the interface between a sensorial organ and a machine (a neuroprosthetic device) which is the cochlear implant.

The significant progress at the national and international level of our team (largely as a result of my activity) provides an expertise base the use of which can be significantly amplified in the benefit of the university by accessing grant programs from a higher teaching position as responsibility and competence.

The main research projects were:

1. Project Partner Coordinator - Research projects won by competition

Microsenzori acustici pe bază de nanofire magnetostrictive pentru aplicaţii medicale – SANAM – contract nr. 12-114/01.10.2008 dintre Centrul Naţional de Management Programe – CNMP Bucureşti şi Institutul Naţional de Cercetare – Dezvoltare pentru Fizică Tehnică IFT Iaşi (PN II, Program 4 - Parteneriate în domenii prioritare), Partener I - Spitalul Clinic de Recuperare Iaşi.

e Centru Naţional de Management Programe – CNMP Bucureşti (Iasi)

2008-10 to 2011-09|Grant

GRANT_NUMBER: 12114

Translated title (Romanian) Acoustic microsensors based on magnetostrictive nanowires for medical applications - SANAM

Total funding amount - RON 2,000,000

Contributors (co-lead)

Description The aim of the project was the development of acoustic sensors based on magnetostrictive nanowires networks, to be used for cochlear implants. Nanowires constitute the active element of the sensor and are stimulated by acoustic signals. Acoustic sensor electrical signal is dependent on the length, diameter, density per unit area and composition of nanowires. It was also tested the role of the membrane, that covers the nanowires, in the sensor response. The project was conducted in partnership with research from two universities and a clinical hospital. The role of our team as partner was to evaluate the biocompatibility of the use material in rat.

Rădulescu L, Mârţu C, Manolache O et al.
The biocompatibility of acoustic micro sensors based on magnetostrictive nanofibers Romanian Journal of Oral Rehabilitation. 2013; 5(2):20-25. (BDI Indexed)

2. Project Coordinator - Research projects won by competition

Optimizarea tratamentului cu implant cochlear la copiii cu hipoacuzie senzorineurală autosomal recesivă nonsindromică cu mutații la nivelul genei GJB2

Acronimul proiectului – OTIC-Gene – Contract nr.25/13M din 19 septembrie 2016 Între Unitatea Executivă pentru Finanţarea Învâţământului Superior și Universitatea de Medicină şi Farmacie „Grigore T. Popa" – Iaşi (PNIII - Cooperare Europeană şi Internaţională , Subprogramul 3.1. Bilateral/multilateral, Apel comun ASM-ANCSI-2016).

Translated title (Romanian) Optimization of the Cochlear implant Treatment in Children with Autosomal-Recesive Non-Syndromic Hearing Loss Due to GJB2 Mutations

Total funding amount - RON 26,865 + lei moldovenesti 200,000

DESCRIPTION Congenital neurosensorial hearing loss is recognized as a major public health problem. Hearing screening allows early detection of newborns with hearing loss, and cochlear implant is the standard treatment. The functional results of the implantation are heterogeneous, without the decisive factors being known. Among the etiological factors there are mutations in the GJB2 gene. The study of the outcomes in cochlear implanted children shows that among them there are both good and bad performers. It follows that mutations in the GJB2 gene are a determinant prognostic factor for implantation results. In this context, we propose to perform a phenotypically complex evaluation of children with mutations in Connexin 26 gene (structure of the ear inner, auditory nerve, vestibular function, associated pathologies, etc.) correlated with the type of implanted electrode, the type of speech processing used, and not least the speech therapy that the child benefited from. The purpose of the study is to identify the factors that mainly determine the results with cochlear implants in order to personalize the treatment (electrodes chosen according to the structure of the cochlea, bilateral implantation function of vestibular damage, etc.) maximizing the child's auditory-verbal rehabilitation. Our final goal is to create a multidisciplinary team for the investigation of genetic deafness and to find a molecular-genetic diagnostics algorithm. The study group is represented by children with nonsndromic deafness. Under the methodological approach, the study will be conducted on three levels: epidemiological, clinical and molecular-genetic. There will be also a control group to assess the spectrum of mutant alleles in the normal hearing population. The clinical plan consists in determination of the phenotype and the particularities of the implant fitting. The molecular-genetic plan seeks to develop an algorithm that will allow the genetic diagnosis. The project will be conducted in partnership with researchers from the University of Medicine and Pharmacy from the Republic of Moldova.

Radulescu L, Curocichin G, Buza A, Parii S, Meriacre T, Chiosa DC, Butnaru C, Birkenhaeger R, Martu C.
Efficiency of SNPs for the Detection of 35DelG Mutation in 50 Cases with Nonsyndromic Hearing Loss, Revista de Chimie. 2018; 69(8): 2273-2277. (IF=1.605)

NATIONAL AND INTERNATIONAL RECOGNITION

1. Scientific quality and visibility of scientific production (international prizes, lecture invited at leading conferences, etc.)

Visiting Professor of the State University of Medicine and Pharmacy "Nicolae Testemițanu" of the Republic of Moldova.

This year – 2019 - I was invited to be an honorary member of the Danubian ENT Society.

Lecturer invited at leading conferences (selection):

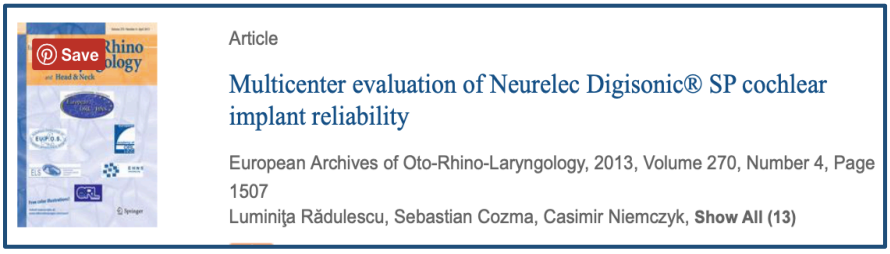
1. European ORL Congress Vienna, 30 June- 4 July 2007
   Round Table: Treatment in Sudden Hearing Loss – Controversies in Europe Inner Ear
1. *Conventus Societas ORL Latina - Lecce, 2010*
   Instructional course: Bilateral Cochlear Implantation with Single Device - Concept, Device Presentation and Surgical Technique
   Round Table: Deafness Neonatal Screening and Cochlear Implant
2. 10th European Symposium of Paediatric Cochlear Implantation (ESPCI) Athens, 12-15 May 2011.
   Chairperson: Round Table -Cochlear implantation in Central and Eastern European Countries
   Panelist - Cochlear implantation in teenagers. Success or disaster)
3. European ORL Congress Praha, 7-10 June 2015
   Round Table: Chronic Serous Otitis Media
4. *Conventus Societas ORL Latina. Torino, 2016*
   Panelists: Impianti cocleari nell'anziano: il limite si sposta sempre di più?
5. The 13th ORL Danube Symposium – Cluj, 2016
6. National Conference with International Participation – Chișinău, 12th of May 2017 – Certificate Seria MORL, cod XVII, no. 1495. (Issued by Ministry of Health of the Republic of Moldova)
7. 13th European Symposium of Paediatric Cochlear Implantation (ESPCI) Lisbon, 25-26 May 2017.
   Chairperson: Plenar Session 4: Cochlear Implants Through the Crystal Ball
   Panelist: RT 13 Access to cochlear implants around the world
8. Conventus Societas Orl Latina - Sinaia, 2017
   Panelist - Conductive hearing loss . Difficult cases. Treatment options
   Moderator: Round Table – Cochlear implant - why two?
9. Coordinator of the Cochlear implant section of 42nd Conventus Societas Latina – Sinaia, 6-9 September 2017 – email from the President of the Congress.
10. 31st Politzer Society Meeting / 2nd Global Otology Research Forum – Las Palmas de Gran Canaria, 21st – 24th of February 2018 – Certificate signed by the Presidents of the Congress (Prof. Angel Ramos, Prof. Manuel Manrique)
11. Invitation to give an Instructional Course at European Academy of Otology and Neurotology) EAONO 2018 in Copenhagen, 20-23 June 2018 – email signed by the Secretary and President (Martin Nue Møller, Per Caye-Thomasen)
12. The 14th ORL Danube Symposium – Krems (Austria), 20-23 June 2018 – letter of invitation
13. VIII Congreso Iberoamericano de Implantes Cocleares y Ciencias Afines – Pamplona, 5-8 de Junio 2019 – Letter signed by the Presidents of the Congress (Prof. Angel Ramos, Prof. Manuel Manrique)
14. National Conference with International Participation – Chișinău, 27th of April 2018 – Certificate Serial MORL, cod XVII, no.4. (Issued by Ministry of of Health of the Republic of Moldova)
15. Balkan Congress of Cochlear Implant – each year
16. Balkan Congress of ORL – Montenegro, 2014.

Reviewer - Medical Principles and Practice journal, IF =1.103

2. Awarding of research results

Awarding of research results by UEFISCDI (Executive Agency for Higher Education, Research, Development and Innovation Funding):

First author:
Radulescu L, Cozma S, Niemczyk C, et al. Multicenter evaluation of Neurelec Digisonic® SP cochlear implant reliability. European Archives of Oto-Rhino-Laryngology. 2013, 270(4):1507-12. (IF =1,63).

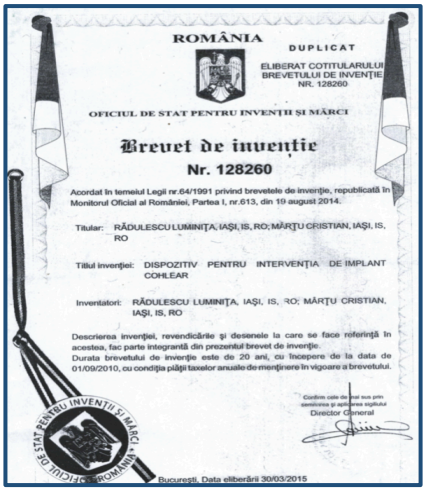


Co-author:
Corodeanu S, Chiriac H, Radulescu L, Lupu N. Magneto-impedance sensor for quasi-noncontact monitoring of breathing, pulse rate and activity status. Journal of Applied Physics. 2014,115(17):301-3 (IF=2.32).

Patent /brevet:

Device for cochlear implant surgery.

Patent No 128260
Published in the Official Monitor of Romania,
Part I, no. 613/19 Aug 2014.



Distinctions:
Certificate of merit:

- National Society of Family Doctors
- University of Medicine and Pharmacy "Nicolae Testemițanu" Chișinău

3. Scientific independence

Scientific event's organizer:

- Vice-president of National ORL Congress – Iași, 2010
- Co-president of Paediatric National Congress – Iași, 2016
- President of the 15th Balcanic Congress on Hearing Implants and High-Tech Hearing Aids – Iași, 2017
- **President of the 14th European Symposium of Paediatric Cochlear Implantation – Bucharest, 2019.**

Research Projects:

International Project Coordinator:

- Optimization of cochlear implant treatment in children with nonsindromic recessive neurosensory hearing loss secondary to GJB2 gene mutations, OTIC-Gene / Contract No. 25BM / 2016 of the ASM-ANCSI-2016 call – won by competition.

Partner project manager:

- Microsensors based on magnetostrictive nanofibers for medical applications - SANAM - contract no. 12-114 / 01.10.2008 between the National Center for Programs Management - CNMP Bucharest and the National Institute for Research and Development for Technical Physics IFT Iaşi (PN 11, Program 4 "Partnerships in Priority Areas"), Partner 1 - Iaşi Clinical Rehabilitation Hospital - won by competition.

Member in the research team:

- OSVaLD – Three-month observational study in patients with recurrent peripheral vestibular vertigo to evaluate the effect of Betahistine 48 mg / day on the quality of life and symptoms of dizziness - 2004 starting date - clinical investigator. Direct assignment.
- Validation study of normal hearing children in Romania - LittlEARS questionnaire of 12 July 2005, PN 2005PMS008, sponsored by MED-EL electromedizinische Gerate GmbH - participant - 2005-2006. Direct assignment.
- Micro and nanoparticles based on drug-containing polysaccharides for the treatment of some eye and respiratory diseases "- contract CNCSIS 258 / theme 31/2007 – won – national competition.
- Implantable magnetic microsensors for medical applications (MEDISENS) – Parteneriate in domenii prioritare – Contract nr. 12-110/01.10.2008. – won national competition.
- Novel particulate drug delivery systems targeting the posterior segment of the eye Call name: Joint Applied Research Projects - PCCA 2013 – call PN-II-PT-PCCA-2013-4-1570 2014 – 2017- won – national competition.
- Optimal therapy protocol for deaf newborns with auditory neuropathy studying electroneural and audiological dynamic progress during the stimulation by conventional hearing aid and cochlear implant. Proiect de cercetare obtinut prin competitie interna - 2015 - Universitatea de Medicină și Farmacie ”Grigore T. Popa” Iași Contract no. 31585/23.XII.2015 – won – intern competition.1.950.000 lei.

Coordinator of the National Sub-Program within the National Health Program of Hearing Rehabilitation through Cochlear Implant and other Implantable Prosthesis since 2009 – present.

4. INTERNATIONAL VISIBILITY – IN SUMMARY

International visibility is reflected by Web of Science H-index: 6; All Databases H- index: 7; total number of citations: 112 (one paper has 40 citations); invited speaker (panelist, moderator, chairperson) in more than 17 prestigious scientific meetings; invited professor to University of Medicine and Pharmacy ”N. Testemițanu”, Chișinău, Moldova Republic (Visiting Professor of theUniversity); ISI papers: 22 (of which 10 as main author); articles BDI: 35 (of which 11 as main author); activity in international grants – grant director (1 project), activity in National grants – partner director (1 project), and team member: 3. President of Balcanic Congres of Cochlear Implantation – Iași 2017, President of Paediatric European Symposium on Cochlear Implantation – București 2019.

This year – 2019 - I was invited to be an honorary member of the Danubian ENT Society.

SECTION I

SCIENTIFIC ACHIEVEMENTS

OPTIMIZING CHILDREN'S JOURNEY TO THE HEARING WORLD

INTRODUCTION AND BACKGROUND INFORMATION

Hearing loss / hearing impairment / deafness represents partly or totally inability to hear sounds. More exactly hearing loss is considered whenever the auditory threshold – measured in dB for different frequencies - is raising above 20 dB in one or both ears (Bance M, 2007).

Hearing loss affects more than 466 million people worldwide (between 8 and 10% of the population) being the most common sensory deficit in human. According to statistical data provided by the National Institute of Deafness and Other Communication Disorders (NIDCD), 3 / 1,000 children under 3 years and 3,5/1000 individuals under 19 are hearing impaired. (Morton and Nance, 2006)

To ease the diagnosis, prognosis, and therapy, hearing loss can be classified (Love, 1929; Clark, 1981) according to several parameters:

- depending on severity (mild: 26-40 dB, moderate: 41-60 dB, severe: 61-80 dB, profound: 81+ dB) (Ansel et al., 1999);
- depending on the site of lesion:
  - sensorineural (caused by an inner ear and cochlear nerve)
  - conduction (caused by any anomaly that interferes with the transmission of the sound through the external ear and the middle ear to the inner ear),
  - mixed (is a combination of conductive and sensorineural damage in the same ear).
- depending on the onset relating to language development (which is situated around the age of two years old): prelingual and post-lingual;
- depending on the etiology (Bordley and Hardy, 1951):
  a. genetic:
     - syndromic and non-syndromic
     - according to the mode of transmission:
       - autosomal dominant
       - autosomal recessive,
       - X-linked, and
       - mitochondrial.
  b. acquired (prenatal, perinatal, and postnatal)
  c. idiopathic.

Etiology of deafness

Genetic

- Syndromic forms associate hearing loss with other congenital abnormalities in certain characteristic patterns.

The most prevalent *autosomal dominant* syndromic forms are:

- Waardenburg's syndrome (variable degrees of neurosensory hearing loss, pigmentation of the skin, hair and iris and characteristic facial dysmorphism) (Mouriaux et al., 1999)
- Branchio-oto-renal syndrome (BOR) can involve anomalies of the branchial arch system, ears - sensorineural or mixed hearing loss, and renal agenesis), (Chen et al., 1995; Lindau et al, 2014)
- Stickler's syndrome (progressive neurosensory hearing loss, palatoschisis and spondiloepidiasis) (Niffenegger et al., 1993)
- Type 2 neurofibromatosis (vestibular schwannoma, increased risk of other meningiomas, astrocytomas, ependymomas). (Slattery, 2015)

The most important *autosomal recessive* syndromes are:

- Usher syndrome (neurosensory hearing loss and retinitis pigmentosa) (Keats and Corey, 1999)
- Pendred syndrome (Wémeau and Kopp, 2017),
- Jervell syndrome and Lange- Nielsen syndrome (hearing loss and prolongation of the QT interval on ECG leading to syncopal episodes and even sudden death) (Eftekharian et al., 2015)
- Refsum disease (peripheral polyneuropathy, cerebellar ataxia, severe neurosensorial hearing loss, retinitis pigmentosa and ichthyosis) (Wanders et al.,1993).

X-linked syndromic hearing loss includes:

- Alport syndrome (progressive neurosensorial hearing loss, glomerulonephritis and ophthalmic anomalies) (Rheault, 2012).
- Mohr-Tranebjaerg syndrome (hearing loss, dystonia, optic nerve atrophy) (Binder et al., 2003).

The mitochondrial hearing loss includes:

- Kearns-Sayre syndrome (progressive external ophthalmoplegia, retinitis pigmentosa, cardiac failure and hearing loss) (Khambatta et al., 2014).
- MELAS (hypoesthesia, encephalopathy, hearing loss, myopathy, gastrointestinal symptoms) (Zwirner and Wilichowski, 2001).
- Recent studies have shown that the mitochondrial transmission of hearing loss plays an important role in the genetic susceptibility to the ototoxicity of aminoglycosides, and hearing loss is a consequence of this, caused by genes that do not necessarily express the cochlea (Gao et al., 2017).

- Non-syndromic forms - Over 127 mutations are involved in non-syndromic hearing loss, some of which are also responsible for syndromic hearing loss. The most common mutations are: GJB2, GJB6 and GJB3 (Van Camp, 2018)
  - The mutation of the GJB2 gene (connexin 26) is responsible for approximately 50% of non-syndromic autosomal recessive hearing loss, but also for some dominant autosomal syndromic forms, being the most frequent cause of pre-lingual hearing loss. The hearing loss caused by the GJB2 mutation is usually bilateral and nonprogressive, with more than 80% of the cases being severe or profound (Feldman et al., 2004)
  - Other genes involved relatively frequently in non-syndromic deafness are GJB6, GJB3, SLC17A8, MYH14, DFNA5, WFS1, TECTA, POU4F3, MYO7A (Liu et al., 1997; Liu et al., 2000; Young et al., 2001; Moteki et al., 2012; Freitas et al., 2014).

Acquired

- Infections during pregnancy: rubella, toxoplasmosis, epidemic parotitis, influenza and cytomegalovirus infections cause sensorineural hearing loss in the child.

- Secondary to the action of toxic substances: thalidomide, quinine and aminoglycosides produce multiple abnormalities (Graydon et al., 2019).

Idiopathic

- Sudden sensorineural hearing loss is rare in children. Up to 90% of SSNHL is idiopathic at presentation and is presumptively attributed to vascular, viral, immunizing, or multiple etiologies (Rauch, 2008; Qian et al., 2018).

Onset of hearing loss relating to language development

- Prenatal deafness may be due to genetic factors and also may be acquired (secondary to prenatal hypoxia, premature birth, and fetal erythroblastosis).
- Postnatal deafness - are also genetic with late onset or acquired. The most common causes are infections - meningitis and meningo-encephalitis, parotitis, measles, otitis media, ototoxic drugs, etc.

Ninety percent of hearing losses are sensorineural, and surgical and medical corrections are much more challenging than in conductive hearing loss.

Diagnosis of hearing loss requires collaboration between the ENT physician, audiologist, geneticist, ophthalmologist and pediatrician. The patient requires a thorough clinical examination, audiometry, CT scan, MRI, cytomegalovirus screening, molecular genetics tests and so on (Sininger, 2003; Kachniarz et al., 2015).

Normal hearing at birth is one of the prerequisite conditions for speech acquisition. In this context, early identification and treatment of deafness (the most frequent sensorial pathology encountered at birth – 1-3‰ of newborns), represents one of the most important preoccupations for the public health policy in the developed countries.

Neuroscience research indicates optimal periods for auditory development and, consequently, the importance of maximizing early hearing experience by early cochlear implantation in children with limited residual hearing.

Nowadays, speech development reports show better results for infants implanted very early, bringing evidence that deaf children under 12 months benefit most from cochlear implantation. Early intervention provides better language development due to maximum cortical plasticity in the early years of life.

Granier-Deferre, described in 1995 that the human cochlea is functional at birth (Granier-Deferre et al., 1995), but in 2005, Ani Sharma showed that the auditory system is still immature and undergoes many developmental changes postnatally (Sharma et al., 2005). This postnatal development is reflected in age-related changes in cortical evoked potentials. For example, the cortical P1 response systematically decreases in latency with increasing age. (Sharma et al., 1997; Cunningham et al, 2000 and Ponton et al, 2000). Prelingually deaf children implanted under 3.5years of age had age appropriate P1 latencies within 6 months following the onset of stimulation. In contrast, P1 latencies for children implanted after age 7 were delayed comparing to normal-hearing controls even after years of stimulation.

In 2016 Andrej Kral and colleaques observed the fact that the brain extensively adapts to the early sensory loss, leading to sensitive developmental periods (Kral A et al., 2016). The compensation of cortical deficits by neurosensory prostheses is subject to an early critical period. Factors of outcome variability can be genetic, biological, cognitive and social. Auditory deprivation has widespread effects on brain development, affecting the capacity to process information beyond the auditory system. After sensory loss and deafness, the brain's effective connectivity is altered within the auditory system, between sensory systems, and between the auditory system and centers serving higher order neurocognitive functions. As a result, congenital sensorineural hearing loss could be thought of as a connectome disease, with interindividual variability in the brain's adaptation to sensory loss underpinning

much of the observed variation in outcome of cochlear implantation. Different executive functions, sequential processing, and concept formation are at particular risk in deaf children.

My attention was directed toward ”*Early diagnosis and treatment of deafness in children”* because the diagnosis and treatment of congenital hearing loss in children has several particularities and also many unanswered questions.

Progress in medical technology has created new opportunities in the treatment of deafness, allowing us to prompt intervention. In the last year the concepts of personalized treatment and of holistic treatment of deaf child imposed itself.

Thus, there are four main medical conduct pathways and I had participated in the development of each of them:

1. The first is **early detection and intervention** of children with hearing loss using electrophysiological methods (otoacoustic emissions – OAE, brainstem evoked response audiometry – BERA, auditory steady state response – ASSR). Such methods lead to the diagnosis of congenital deafness from the first days after birth (Gorga et al., 2005). They are non-invasive, relatively easy to perform and are highly available for usage as screening tools for deafness in maternities (Norton et al., 2000). The hearing loss screening has to be followed by positive audiological diagnosis.
2. The second pathway consists of **molecular diagnosis** used to establish the etiology of deafness in some countries (Alves and Ribeiro, 2007; King et al., 2012)
3. The third pathway takes into account the **new possibilities of deafness treatment** either with powerful conventional digital hearing aids or with implantable prosthesis for the middle ear (like BAHA - bone anchored hearing aid), for the internal ear (cochlear implant) or for the eighth nerve (brainstem implants) (Davis, 1997; Tharpe and Gustafson, 2015).
4. Last but not least **- personalized fitting and speech rehabilitation** with direct input from all members of the family along with speech therapists and audiologists.

The success story of the congenital profound deaf children that overcome deafness began in early 60s when doctor William House learned that his colleague from France doctor Charles Eyres together with the electrophysiologist Andre Djourno succeeded in 1957 to produce a sensation of hearing by placing an electrode in contact to the eight nerve in a patient operated for a huge cholesteatoma that destroyed the inner ear.(Djourno and Eyries, 1957). Based on the French experiments doctor House began his work in the field of cochlear implantation. It was in 1961 when together with Jim Doyle he implanted the first ever cochlear implant (CI) with one channel in Los Angeles. (House, 1973) Later on, some other teams especially from Germany and US joined the CI research. It was the experimental period of CI.

The second period began in 1970 and was the trial period when a cohort of patients has been cochlear implanted (Mudry and Mills, 2013). In 1973 during the First Conference on Electrical Stimulation of the acoustic nerve as a treatment for the profound sensorineural deafness in human, organized in San Francisco the term ”cochlear implant” was introduced in medical literature. In the same period the multichannel single wire electrode was initiated by Clark in 1978 (Clark et al., 1978) and Simmons and White in 1979 (Simmons et al., 1979). Their studies launched the third period - that of commercialization. In this third stage, other teams from Germany, Belgium and Austria started their experiments (Hochmair et al., 1981; Battmer et al., 1985).

In 2000 – after 20 years from the beginning of the Commercial period, a surgical team made up of Professor Dan Martu and I implanted the first patient in Romania under the patronage of UMF Iasi.

In 2004 our Clinic entered the National CI Program. In 2007 in Rehabilitation Hospital Iasi the Cochlear Implant Compartment was created and in the same year I was designated head of the Compartment.

After numerous claims, in 2008 our Clinic started to coordinate the Hearing loss screening program in newborns in Iasi.

In 2008 I became responsible of the Cochlear Implant Program in Iasi.

Hearing loss rehabilitation in children is essential to ensure the proper development of language, speech, and social skills (White et al., 2019). The success of cochlear implantation in congenital profound deaf children depends mainly on early identification - meaning until 3 months of age, followed by early intervention by fitting hearing aids up to 6 months of age and if the child has no benefit from the hearing aids - cochlear implantation have to be done by 12 months of age.

The experience accumulated In the years that follow the first cochlear implantations in children, shows that the concept of early detection and early intervention is critical for hearing loss rehabilitation, thus the 3:6:12 months rule was gradually replaced by the 1-3-6 rule (screen for hearing loss by age 1 month, complete a diagnostic audiologic evaluation by age 3 months, and enroll in appropriate early intervention services by age 6 months) this last rule being best suited for the child (White et al., 2019).

The first goal for a successful cochlear implant program is early identification of congenital severe to profound deaf children. This can be accomplished with national hearing screening programs. Healthcare systems worldwide have developed over years several types of programs to address deafness and improve the quality of life of people with hearing loss - among these we mention Newborn hearing screening programs (universal or not), aiming at detecting hearing loss from birth, which is one of the most frequently occurring permanent congenital defects and interferes with typical communication development. The prevalence of significant bilateral hearing loss at birth is 0.1–0.3 % for newborns and 2–5 % in presence of audiological risk factors (Ohl et al., 2009; Speleman et al, 2012; Thangavelu et al., 2019)

Deafness in children has heavier consequences with regards to auditory perception, but also speech and language development as well as scholarship.

Neonatal hearing screening for congenital sensorineural hearing loss meets all the criteria for universal screening:

- high incidence of hearing loss at birth - greater than the combined incidence of all the metabolic conditions that are currently screen for with blood tests.
- with serious consequences for the affected child and his family, as hearing during the critical periods of infancy is a fundamental prerequisite to develop spoken language unless early and appropriate management.
- there are available technologies (otoacoustic emission and automated auditory brainstem response testing): accurate, reliable, easy to be performed, objective and, cost-effective to detect congenital hearing loss.

Most of the developed countries have started screening programs (Geers A, 2006; Lévêque M et al., 2007). In 2007, the Joint Committee on Infant Hearing (JCIH, 2007) released a unique list of risk indicators associated with congenital/neonatal hearing loss and delayed-onset/acquired or progressive hearing loss. The JCIH recommends monitoring hearing, and speech and language skills of all infants as well as performing an audiological assessment at least once by 24–30 months of age in infants presenting with one or more risk indicators from this list (JCIH, 2007).

Early detection through newborn hearing screening and hearing technology provide most children with the option of spoken language acquisition. Late diagnosis of hearing impairment may negatively result in child's language, cognitive, social, emotional, and academic development. The hearing loss itself and its mentioned negative results may significantly affect the child's quality of life. Universal Neonatal Hearing Screening (UNHS) programs were developed in several countries to identify the majority of newborns with hearing impairment. UNHS programs adopt, as screening tests, otoacoustic emissions (OAEs) and/or automated

auditory brainstem response (aABR) testing. Those who are positive at tests are referred to full audiological diagnosis. Audiological or medical/surgical management, educational and (re)habilitation methods, and child and family support are available strategies for subjects with confirmed hearing loss (JCIH, 2007).

Recognized benefits of UNHS are: better language outcomes, better speech discrimination at school age and improved long-term language development, as well as their socio-emotional development. Therefore, universal newborn hearing screening is widely recommended and implemented by governments or mother and child health agencies.

Since 1999, process and outcome performance indicators and benchmarks were established for Early Hearing Detection and Intervention (EHDI) programs (i.e., screening before 1 month of age, identification before 3 months and intervention by 6 months) to evaluate progress and determine consistency and stability (Grindle, 2014).

Nevertheless, in the European Union, there is a huge variability in the introduction of universal vs non-universal screening, in the organizational screening process, as well as in guidelines referring to screening test technologies, tracking process, and rehabilitation. Guidelines translate best evidence into best practice (Vos B et al., 2016). A well-crafted guideline promotes quality by reducing health care variations, improving diagnostic accuracy, promoting effective therapy, and discouraging ineffective – or potentially harmful – interventions. For instance, one key issue for screening is the problem of false-negative screening tests and delayed onset (Vos B et al., 2016).

A particular attention must be paid to severe to profound hearing loss.

Children with severe to profound hearing loss will be focused on since they are the patients with the highest societal costs among overall hearing loss. In the US, life time costs for an individual with severe to profound hearing loss with prelingual onset exceed USD 1 million (Mohr et al., 2000). Children with severe to profound hearing loss are the population with the heaviest consequences in terms of societal outcomes (communication, scholarship). Rehabilitation needs expensive therapeutics based on a class-III active medical device, which needs systematic evaluation because of the variability of outcomes, technology evolutions, and inherent associated risk of adverse events (device failures and complications).

The etiology of hearing loss is genetic in the majority of cases and although the cause is genetic, the hearing loss might have a late-onset or might be progressive and to fail to be identified by conventional newborn hearing screening (Wu et al., 2011). Genetic screening for deafness might compensate for the inherent limitations of conventional universal neonatal hearing screening.

One of the aims of our international research project (project that was won by competition in 2016) was to determine the etiology and to increase knowledge of the genetic causes of hearing loss in our region and then to find a reliable, cost-effective method to perform the genetic screening of hearing loss. We also have considered and discussed ethical implications of genetic screening (Tarini and Goldenberg, 2012; Linden Phillips et al., 2013).

I.1. OPTIMIZING EARLY DETECTION OF DEAF CHILDREN

I.1.1 Neonatal screening of hearing loss in newborn

Background

The incidence of congenital hearing loss is between 1 – 3 ‰ of newborns exceeding the incidence of any other congenital illness (Morton and Nance, 2006). Any degree of hearing loss has an important impact on speech acquisition and cognitive development of the child. A near normal speech and language rehabilitation as well as a normal cognitive development of the child are possible when the detection and intervention are subject to the rule of:

- detection – by first month of age
- diagnosis - by three months
- intervention – by 6 months consisted (for the sensorineural deafness) in prescription of a hearing device and speech therapy. The results are still good if the intervention is done in the first 2 years of life – these first 2 years are considered to be the critical period for language acquisition (Yoshinaga-Itano, 2013).

Deafness is an invisible and painless illness, so detection of the deaf born children is possible by systematic testing of all babies immediately after birth. The universal neonatal hearing screening represents the solution at this issue.

Personal contribution

Scientific and professional achievements:

Within this direction of study concerning optimizing early diagnosis of children with congenital deafness, study performed by our entire medical team from Rehabilitation Hospital and from Maternities, we have published one original research article, in a journal indexed Copernicus. This study was of major importance in deciding the National Hearing Screening Protocol which entered in force this year.

Published paper:
Cozma S, Mârţu C, Manolache O, Olariu R, Damean G, Cavaleriu B, Zota D, Mârţu D,ț **Rădulescu L**. 6 ani de screening auditiv neonatal universal la laşi - rezultatele unui parteneriat interdisciplinar. ORL.ro . 2015, 8(27):26-31.

Aim of the study

The aim of the study was to analyze and to present the results of the universal neonatal screening conducted in Iași by our hospital over a 6 years period and to establish the gold method of screening in accordance with the specific conditions from our region.

Material and methods

It is a prospective study that included a cohort of 45785 newborns representing 93.45% of total babies born in Iași maternities from January 2009 until December 2014.

In the beginning of the survey there were used for hearing loss screening two complementary methods (otoacoustic emission and automated auditory brainstem response testing) in both maternities.

The protocol was changed after a while during the first year of screening using further just the otoacoustic emission test.

Results: As a result of the testing the newborns were separated in two groups:

- the first group includes children that do not pass the hearing test and those that have risk factors for deafness.
- the second group consists of babies that pass the screening and have no risk factors.

The babies in the first group were advised to attend specialized service of audiology from Recuperare Hospital to confirm as early as possible the diagnosis of hearing loss or to be discharged if the diagnosis in invalidated.

In the specialized service the children were evaluated at 1 month of age, at 3 months and at 6 months using a diagnosis battery consisting in:

- history
- ear examination
- impedancemetry
- otoacoustic emission
- BERA
- Auditory Steady State Response (ASSR)
- behavioral tests

Subsequently due to the numerous numbers of children the visit at the age of the first month was canceled.

Disscusions

During the 6 years study there were found 67 children with sensorineural hearing loss. The incidence of hearing loss in our region was 1.46‰ of newborns same as the rates reported by the majority of the EU countries (1.5‰) (Caluraud, 2015; Vos et al., 2016).

Both proposed testing methods otoacoustic emissions and automatic BERA are complementary. They are reproductible, objective and easy to be performed, being suitable for the rapid detection of hearing loss. They have to be use together when the intension is to test the entire auditory pathway.

The automatic BERA is an electrophysiological method that offers a qualitative evaluation of the entire auditory pathway. The result is express as PASS when the hearing threshold is normal or REFER if a degree of hearing loss is detected.

The otoacoustic emissions (OAE) is a test described in 1978 by David Camp. The test principle consists of recording the sounds produced by the normal outer hair cells in the cochlea. They are absent if the outer hair cells are damaged.

In recent years it has been observed conditions when the OAE were present and BERA responses were abnormal or absent. The disorder was labeled as Auditory Neuropathy (Starr et al., 1996) or Auditory dys-syncrony and is secondary to the lesions of the inner hair cells, of the axons or of the dendrites of the auditory nerve. The incidence of auditory neuropathy is quite important – representing 8% of the congenital deafness - hence the recommendation to use both tests (OAE and automatic BERA) in the neonatal hearing screening.

In the first year of screening, both tests were used for a while, but due to administrative problems, the automatic BERA test was dropped while maintaining OEA testing.

With the exception of the first year when the rates were quite low (70%) due the necessity to adept the protocol to the maternity′s conditions, in the next 5 years the percentage of the tested babies has reached 98.99%.

The 67 babies were diagnosticated with different types of hearing loss:

34 children were diagnosticated with moderate to severe hearing loss and received hearing aids at the ages between 4 and 8 months.

27 children were diagnosticated with severe to profound hearing loss and cochlear implantation was indicated. All of them were implanted by the age of 24 month.

6 cases were diagnosticated with conductive hearing loss due to malformations of the external and middle ear – they were implanted with a bone conduction hearing device type BAHA (Bone Anchored Hearing Aid).

Among the risk factors of deafness that were found in our study group we have to mention: prematurity, followed by hyperbilirubinemia and children with respiratory problems - usually children coming from neonatal intensive care unit.

A particularity of this cohort consists in the high number of children with risk factors for deafness. This was the consequence of referral of babies with severe health problems to Neonatal Intensive Care Unit Iași from other maternities.

Conclusions

Detection of hearing loss in the newborns it is a feasible task which requires the use of the both proposed testing methods otoacoustic emissions and automatic BERA. These two tests give complimentary results thus all children with hearing loss may be deteceted.

I.1.2. Screening of hearing loss in (children) in early primary school

Background

Present studies show that until the school age the number of hearing-impaired children grows significantly – as a result in many countries campaigns to support pre-school hearing screening are being conducted. Screening of hearing loss in newborns does not solve the problem of hearing impairment during the childhood. Medical literature shows that more than 50% of children diagnosticated with hearing loss in kindergarten or in primary school have passed the neonatal hearing screening (Weichbold et al., 2006; Dedhia et al., 2013).

Even since I was in residency, I participated in a study that was aimed at detecting primary grade school children suffering from hearing loss without having been previously diagnosed.

Personal contribution

Scientific and professional achievements in this field :
Within this clinical pilot study concerning early diagnosis of children with deafness, study performed during my residency, we have demonstrated the importance of hearing evaluation of children in primary school. We have published one original research article, in a BDI journal. This study is important nowadays in deciding the oportunity of Hearing Screening.

Published paper:
Mârţu D, **Rădulescu L**, Mardiros G, Brănişteanu L, Ceauşu C. Screening for hypoacusis in children in grades 2 and 3. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 1996; 100(1-2):146-148.

Aim of the study

The aim of our study was to find how many children in primary school have disabled hearing loss.

Material and methods

At that time and at that group of age, for our screening, we used the otoscopic examination and tonal audiometry. The results were published in Revista Medico-Chirurgicala

a Societatii de Medici si Naturalisti din Iasi. Our study was published in 1996 (Martu et al., 1996) but the subject is now more actual than ever. Nowadays there are a lot of studies that show the importance of hearing screening programs in the school children, although in the majority of developed countries are taking place screening programs in newborns. It is considered that until the school age the number of children with hearing problems increases significantly (Ojha et al., 2016; Yousuf et al., 2018; Dickinson et al., 2018; Cedars et al., 2018*)*

Conclusions

During our study there were some drawbacks but also many achievements:

- The main drawback was that – although initially the screening protocol included both methods of study – it was necessary to renounce to one of them (to automatic BERA) because it was time consuming and many children have remained untested.
- Almost all babies were tested when OAE were used as unique test.
- The most important aspect was that the early intervention was possible in all cases in which hearing loss was found and most children were rehabilitated.
- This pilot study was one of the reference studies for introduction of the universal national neonatal hearing screening in Romania beginning to this year.
- The results of the hearing screening in children from second and third grade school may be also a starting point for hearing screening of children in primary school.

I.1.3 Genetic screening of hearing loss in children

Background

Hearing loss is the most common sensory defect, representing a major public health issue (http://www.who.int/news-room/fact- sheets/detail/deafness-and-hearing-loss).

Causes of hearing loss are both genetic (in 50% of cases) and non- genetic (Denoyelle et al., 1999; Cavaleriu et al., 2015). Non-syndromic genetic hearing loss occurs in 70-80% of cases, the remaining 20-30% are hearing losses occurring during the various syndromes (Koffler et al., 2015; Chang, 2015; Stelma and Bhutta, 2014). Of the total non- syndromic genetic cases, 75-80% are autosomal recessive (DFNB) transmitted, 18-20% autosomal dominant (DFNA), 1-2% X-linked (DFN) and less than 1% by mitochondrial route (Vona et al., 2015; Smith et al., 2005; Bruzzone et al., 1996). In most cases, autosomal non-syndromic SNHL is associated with mutations in the GJB2 gene, responsible for the synthesis of connexin 26 (Cx26). Cx26 mutations are responsible of various human pathologies ranging from hearing loss to keratitis ichthyosis deafness syndrome. Cx26 contribute also to chemosensory regulation of breathing (De Wolf et al., 2016), raising the issue of breathing monitoring during sleep in subjects with these mutations (Corodeanu et al., 2014).

One specific mutation in the GJB2 gene namely 35delG mutation accounts for the majority of the autosomal recessive non-syndromic hearing loss cases in the Caucasian population (Murgia et al., 1999; Chang, 2015). Worldwide efforts are being made to identify biomarkers: for diagnosis, assessment of etiology, risk, as well as personalization of treatment (Zou et al., 2015; Zenner et al., 2014; Radulescu et al., 2007; Perde-Schrepler et al, 2012).

Single nucleotide polymorphisms (SNPs) are important markers in studies that correlate the genotype with the phenotype.

Personal contribution

In 2016 I applied to ASM-ANCSI-2016 – as project director - and won an international research project with the title: *Optimizarea tratamentului cu implant cochelar la copii cu hipoacuzie neurosenzoriala autososmal recesiva nonsindromica cu mutatii la nivelul genei* GJB2, OTIC-Gene / Contract Nr.25BM/2016 din cadrul apelului ASM-ANCSI-2016.

Scientific and professional achievements:

Within this direction of study concerning optimizing early diagnosis of children with congenital deafness, study performed by the team from our University in colaboration with "Testemițanu University", we have published one original research article, in a ISI journal. This study is important from scientific poit of view because proposes an affordable method of great sensitivity and specificity to be used as a screening tool in identification of 35delG mutation and also is important at the institutional level because opens new possibilities of cooperation in research area between Romania and Moldova.

Published paper:
Rădulescu L, Curocichin G, Buza A, Parii S, Meriacre T, Chiaburu Chiosa D, Butnaru C, Birkenhaeger R, Martu C. **Efficiency of SNPs for the Detection of 35DelG Mutation in 50 Cases with Nonsyndromic Hearing Loss,** 2018; Rev Chim; 69(8):2273-2277. (I F in 2018 = 1.6050)

Aim of the study

The aims of the study were:

- to determine etiology and to increase knowledge of the genetic causes of hearing loss;
- to develop and validate a molecular-genetic screening algorithm based on the SNPs with the potential to be later used in the laboratories in Romania and in the Republic Moldova.
- to create a multidisciplinary cross-border nucleus of competence in molecular-genetic diagnosis by co-opting and involving experts on the field of interest.

Materials and methods

The proposed mutation to be screened was based on SNP rs80338939 corresponding to 35delG mutation from GJB2 gene.

The study group consisted of 50 randomly selected subjects with profound congenital autosomal recessive sensorineural hearing loss (SNHL) diagnosed and treated at the ENT Clinic within the Clinical Rehabilitation Hospital in Iasi.

The study obtained the approval of the Ethics Committee of UMF Grigore T. Popa Iasi. Informed consent was obtained from parents or legal guardians for children before collecting venous blood for molecular analysis.

From each child, 6 mL of venous blood was harvested on the EDTA medium.

The 35delG mutation was assessed by two methods:

- to determine etiology and to increase knowledge of the genetic causes of hearing loss and as well as a reference method - the sequential analysis of exon 1 and the coding exon 2 of the GJB2 gene was performed

and

- the method to be validated: single nucleotide polymorphism (SNP) for 35delG mutation (Table I). Extraction was performed using the GeneJET Genomic DNA Purification Kit (K0722, Thermo Fisher Scientific). Analysis of the extracted DNA quality was performed by spectrophotometric method (NanoDrop 2000c spectrophotometer, Thermo Fisher Scientific). Identification of mononucleotide polymorphism was performed by the TaqMan technique. All tests were performed on the QuantStudio 6 flex device (Applied Biosystems, ThermoFisher Scientific). The amplification program, plate design and data collection were performed using QuantStudio Real-TimePCR Software (v.1.3., Applied Biosystems, ThermoFisher Scientific) (Table II).

Table I - Probe used for genotyping

N/o	Name of gene	NCBI SNP Code	Mutation	Probe [VIC/FAM]
1	*GJB2*	rs80338939	35delG Deletion	GGCACGCTGCAGACGATCCTGGGGG[-/G]TGTGAACAAACACTYCACCAGCATT

Table II - Amplification protocol

Stage	Parameters		
	Temperature (°C)	Time	Cycle
DNA denaturation ADN and AmpliTaq Gold® enzyme activation	95	10 min	HOLD
Denaturation	95	15 sec	40
Normalization / Extension	65	1 min	

The main difference between the two methods was that in the method to be validated just a single mutation, 35delG was analyzed using SNP rs80338939.

The sensitivity and specificity of the proposed method for screening test was assessed.

Results

The results obtained after genetic testing in the two laboratories of the 50 subjects with profound congenital SNHL from the studied group are presented in Table III.

To validate the SNP method proposed for screening by the laboratory in the Republic of Moldova, the results from the two laboratories were analyzed comparatively considering the gold standard sequential analysis of exon 1 and the coding exon 2 of the GJB2.

We assessed the sensitivity and specificity of the proposed method (Table IV).

Disscusions

Genetic population studies have shown that the prevalence of GJB2 gene mutations varies according to ethnicity, e.g. in China 16% of cases of hearing loss (Liu, 2002), in Pakistan the prevalence is 6.1% (Santos, 2005), in the population of Iran - 16 % (Ghasemnejad, 2017), 9.6% Mexican (Hernández-Juárez, 2014) and may reach up to 50% in the European population (Kenneson, 2002). Although several mutations of the GJB2 gene are described, in

Table III - Comparative results found with the two different methods

	Molecular Lab Chisinau		Molecular Lab Freiburg	
	ALLELE 1	ALLELE 2	ALLELE 1	ALLELE 2
P1	35delG	35delG	35delG	35delG
P2	35delG	35delG	35delG	35delG
P3	35delG	W	35delG	W
P4	35delG	W	W	W
P5	35delG	35delG	35delG	35delG
P6	35delG	35delG	35delG	35delG
P7	35delG	W	35delG	W
P8	35delG	35delG	35delG	35delG
P9	35delG	W	35delG	W
P10	35delG	W	35delG	W
P11	35delG	35delG	35delG	35delG
P12	35delG	35delG	35delG	35delG
P13	35delG	W	35delG	W
P14	35delG	W	35delG	W
P15	35delG	35delG	35delG	35delG
P16	35delG	35delG	35delG	35delG
P17	w	W	W	W
P18	w	W	W	W
P19	w	W	W	W
P20	w	W	W	W
P21	w	W	W	W
P22	w	W	W	W
P23	w	W	W	W
P24	w	W	W	W
P25	w	W	W	W
P26	w	W	35delG	W
P27	w	W	W	W
P28	w	W	W	W
P29	w	W	35delG	W
P30	w	W	W	W
P31	w	W	W	W
P32	w	W	W	W
P33	w	W	W	W
P34	w	W	W	W
P35	w	W	W	W
P36	w	W	W	W
P37	w	W	W	W
P38	w	W	W	W

Table III (cont.) - Comparative results found with the two different techniques

P39	w	W	W	W
P40	w	W	W	W
P41	w	W	W	W
P42	w	W	W	W
P43	w	W	W	W
P44	w	W	W	W
P45	w	W	W	W
P46	w	W	W	W
P47	w	W	W	W
P48	w	W	W	W
P49	w	W	W	W
P50	w	W	W	W

Table IV – Assessment of the sensitivity and specificity of the SNP test

	35delG present	35delG absent
	ALLELE 1 + ALLELE 2	ALLELE 1 + ALLELE 2
SNP positive	24	1
SNP negative	2	73
	24/ (24+2)	73/ (1+73)
	Sensitivity	Specificity
	0.92 // 92%	0.98 // 98%

the European population the 35delG mutation represents 2/3 of the total mutations in the GJB2 gene (Van Laer, 2001; Gasparini, 2000. In contrast, other mutations predominate in other populations, such as: 235delC mutation in Japanese and other Asian populations (Ohtsuka, 2003), 167delT in the Ashkenazi Jews (Morell, 1998), W24X in Indians and Roma (Ramshankar, 2003; Minarik, 2003).

In North-East Romania and in the Republic of Moldova predominates the 35delG mutation.

One of the expected results of the project was the development of a screening diagnostic algorithm that would allow:

- early diagnosis of children with genetic hearing loss secondary to the 35delG mutation - by highlighting the presence of this mutation on the both alleles of the gene;
- assessing the risk of deafness in brothers and descendants (Denoyelle et al., 1999) and
- early rehabilitation, thus contributing to the development of preventive medicine and personalized curative medicine (Zenner et al., 2014; Martu et al., 2016).

Universal hearing screening using OAE, as it is planned, will statistically result in either false negative and also false positive results, as it is recognized that the sensitivity of TEOAE for identifying hearing loss is around 66.7% and its specificity is 98.8% (Yousefi et al., 2013). Some authors have published some cases that show that deafness due to 35delG mutations may have a late onset and consequently the diagnosis may be missed on neonatal screening

programs. This may be an argument to consider neonatal screening for GJB2 mutations in order not to miss these late onset cases that cannot be identified at birth (Pagarkar et al., 2006; Norris et al., 2006).

The most common GJB2 anomaly in our regions is the deletion of one guanine within the six-guanine string at the beginning of the second GJB2 exon (positions 30-35), the so-called 35delG mutation (rs80338939) (Estivill et al.,1996; Green et al., 1999).

Our study shows the potential of SNPs tests as a neonatal screening tool to identify deaf children in 100% of cases. The late onset of hearing loss could be improved. We also found a high specificity (98%) and sensitivity (92%) of our test for the carriers thus the test could be used with good results to assess the risk of deafness in brothers and descendants. This means that using SNP rs80338939 screening the rate of false results will be much lower:

- a false positive result in 2% of the cases - so 2 subjects out of 100 will be falsely diagnosed as carriers of 35delG mutation and,
- a false negative result, obtained in 8 subjects out of 100 meaning that 8 patients would be erroneously diagnosed free of 35delG mutation;

Using SNP testing the detection of persons with both alleles affected was correct in 100% of the cases.

The false positive and false negative results were obtained only in carriers.

However, it should be stressed that 35delG mutation screening does not replace audiological screening tests because it is thought that up to 1% of human genes are necessary for hearing, and mutations in any of these may lead to hearing impairment.

Conclusions

In conclusion, the great sensitivity and specificity of the proposed method recommends this technique to be used as a screening method to identify 35delG mutation that in homozygous form is an indicator of deafness and in heterozygous form is a sign of being a carrier of a recessive genetic variant.

Further directions:

Our study confirms that rs80338939 can be used as a biomarker in the assessment of the risk of autosomal recessive SNHL. In fact, we propose that in the next step, we optimize the technique to achieve 100% sensitivity and specificity and also to include even more SNPs so in the end we can test all the spectrum of mutations in our region.

I.1.4 Ethics of genetic screening

Background

Molecular diagnosis is used to establish the etiology of deafness in some countries. Molecular screening in children with hearing loss followed up by genetic counseling and eventual prenatal molecular diagnosis in siblings is pending. Genetic mechanisms also seem to be involved in the pathogenesis of deafness caused by middle ear inflammatory diseases. Pediatric cholesteatomas are usually more aggressive and invasive; as demonstrating in studies genes important in inflammatory processes (for example, KRT6B, SPP1 and S100A7A) are highly up regulated in cholesteatoma (Maniu et al., 2014). The A1555G mutation in the mitochondrial RNA gene has been associated with aminoglycoside induced hearing loss (Moroti et al., 2009).

New and improved standards of treatments in different diseases have become available as technology progresses. Thus, DNA decoding created the possibility for prenatal diagnosis in different diseases like deafness by identifying the gene mutations responsible for hearing impairment, such as partial or total loss of hearing (Sugata et al., 2002; Hone and Smith, 2003; Schade et al., 2003; Abe et al., 2007). Genetic mutations are encountered in 60% of congenital deafness. Molecular diagnosis of deafness in families with hearing impaired children could predict the probability of a mutation able to be transmitted to other members of the patient's family, being the reference point for genetic counseling and, eventually, for prenatal diagnosis of deafness, at the parent's request (Coviello et al., 2004; Marpeau, 2008). This approach opens a real possibility for deafness eradication.

Personal contribution

Scientific and professional achievements:

Within this direction of study concerning ethics of genetic tests in children, we outlined the ethical limits in which we can perform genetic testing in children. We have published one article, in an ISI journal. This study was important as it helped us to understand the advantages and limitations of genetic screening for deafness and to improve the informed consent for the genetic testing of cochlear implanted children.

Published paper:

Rădulescu L, Mârțu C, Rădulescu T, et al. Genetic Screening of Deaf Children: Ethical Considerations. Revista de cercetare si interventie socială. 2018; (60):180-187.

Ethical challenges in the genetic management of deafness

In the context of molecular screening of hearing loss some questions arise:

- Is it ethical to forbid the birth of a deaf child?
- Can we integrate deafness into a culture, or should we consider it to be a disability?
- Is it ethical to use genetic information regarding the birth of a child according to his/her hearing condition?
- It is well known that there are deaf people who have the desire to give birth to a deaf child?
- Is the cultural identity of deaf people in danger of disappearing?
- Is it necessary to have the informed consent for molecular diagnosis of deafness?

Variability is a prerequisite of evolution. This law of biology leads to the evolution of species by natural selection. To be different from normal can offer unexpected and unknown advantages to a human being or to a group of people provided the difference is not a disadvantage. Evolution regarding human life in modern society with developed medicine is an abstract concept.

Further on, we may ask ourselves what direction the human species will follow – giving way to diversity including evolution without interfering with the human genome or having it under control and guiding it towards perfection. Having diversity in view, one can ask if deafness has any advantages. It is known, for example, that sickle cell anemia provides some resistance to malaria. It is also a fact that people with Down syndrome are protected from some forms of cancer (Hasle et al., 2000).

Are there any advantages in being deaf? Is it ethical to give birth to people with this disability (or to do nothing in order to prevent their conception) just for the sake of a natural experiment? The immediate benefit and good health should be the moral guidelines. Can we integrate deafness into a culture, or should we consider it to be a disability? Authors like Johnston T. (Johnston, 2004) supported the view that deafness is not a disability. Those who support the deaf culture say that deafness unlike other disabilities has its own language – sign language – therefore creating, in turn, a linguistic community. Having this in view, deafness is not a disability (Johnston, 2004; Padden and Humphries, 1988).

As a result, the pathological concept is inadequate and the only way to understand the status of deafness is to be deaf yourself. In a study from 2001 conducted by the Study Center of Deafness in Bristol it is shown that the majority of deaf persons consider deafness as a disability (Dye et al., 2001). From the point of view of a deaf child it is not fair that other children have a sense they do not have. In such a context, persons who can hear are cheaters. If we could choose between being deaf and having the sense of hearing, what would be our option? The advantage of being able to hear or the lack of this capability? The study carried out in Bristol (Dye et al., 2001) certifies that this conclusion is valid for a large number of deaf people. Asking a deaf person, "would you rather hear?" has no sense and could not have any answer. It is not possible to limit deafness to the absence of only one sense. A deaf person cannot imagine being something he has never experienced. The life of a deaf person may be empty or like the one of any other persons who can hear.

For a child with deaf parents – this disability is virtually nonexistent in the first part of his/her life and in the beginning life experience can be normal. Later on, communication with persons with normal hearing, hence with the whole society, becomes a problem and deafness creates limitations and difficulties as far as life opportunities are concerned. Is it ethical to intend to have or to determine the birth of a child who will be deaf?

If deafness is considered not to be a disability, then there are no ethical problems for the person who wants to have a deaf child. But, as the majority of people consider deafness to be a disability, an ethical dilemma might arise. In Nazi Germany deaf persons were not allowed to get married, such persons were forced to be sterilized or were even killed (Schuchman, 2004).

Advantages and limitations of genetic screening for deafness

Today the situation is totally different, hearing loss screening and early treatment make it possible for a person to be able to hear. Bioethics studies evaluated the moral values and concepts to be included in decision making. In a complex moral universe, a moral code should secure maximum personal happiness for everyone. In the universal moral code, a minimum standard should include the principles of not harming and of being good (the principle of beneficence). According to the highest principle of autonomy the patient is in the most favorable position to obtain all that can satisfy him and make him happy as an individual. This principle cannot be applied to a young child because his parents are the ones who should decide for him. It is to be understood that each parent wants what is the best for his child. Therefore, a parent cannot desire (according to the principle of beneficence for his child) to give birth to a child with a predictable but avoidable disability.

However, if the parents do not perceive deafness as a disability, then their desire to have deaf children is to be understood (Murray, 2004). If we accept that deafness is not a disability the position held by such parents can be ethical. On the other hand, it is not necessary to create deaf children just to perpetuate the culture and language of the deaf people.

In countries with advanced medicine, the deaf community is shrinking and therefore the desire to have deaf children to preserve the community is increasing. Nevertheless, to secure a culture based on a disability cannot be justified. Do parents have the right to select their children based on their hearing status? The human biological right implies five intact and functional

senses at birth. With this requirement, the conception of a child with limited sensorial capacities is a violation of this human biological right. We do not have the right to choose deafness, taking into account the future consequences for the child.

Progress in genetics has allowed widespread use of genetic tests that can be used to detect deafness. Using them to prevent the birth of a deaf child has created ethical controversy between the deaf community and the medical world.

While physicians view deafness as a curable disability, Deaf community members perceive deafness as a culture and so treatment of deafness through healing would lead to their disappearance (McKee et al., 2013).

Genetic tests allow avoidance of giving birth to a deaf child by simply selecting a partner. The increase of deafness through connexin-26 in the last 200 years in the US has been attributed to marriages between people with the same genetic defect (Nance et al., 2000).

Middleton and others studied the preference of having normal hearing or deaf children based on prenatal genetic tests, to allow the diagnosis of deafness, in a group of 87 deaf adults in England, and then in a group of over 1300 deaf. The study showed that deaf persons are not interested in prenatal diagnosis of hearing loss (Middleton et al., 2001). A feature of those with profound deafness is the marriage based on linguistic monogamy (McKee et al., 2013).

Stern and his colleagues examined the attitude of the deaf in the US on a group of 337 persons using a questionnaire similar to that used by Middleton (Middleton et al., 2001; Stern et al., 2002). The results were similar. Although they had a positive attitude to genetic testing including prenatal testing, no one wanted to use such information to prevent the birth of a deaf child (Stern et al., 2002).

Brunger's studies show the same situation: most deaf people have a positive attitude towards genetic testing, but no one wants to use the information to stop the pregnancy. The study was performed on a group of 96 patients whose children were deaf (Brunger et al., 2000).

Martinez points out differences in attitude between normal hearing persons, profound deaf and deaf-mute persons in terms of their attitude to genetic testing. The study was conducted on 133 normal hearing persons, 60 deaf-mute and 29 persons with profound deafness. However, most agree with testing newborns rather than prenatally testing for deafness (Martinez et al., 2003).

The study conducted by Taneja and collaborators evaluated the attitude of the cultured deaf individual (students at an art university) regarding the genetic testing of newborns and also the attitude regarding the use of genetic tests for choosing a partner for marriage. The study was conducted on 77 students over 18 years. Regarding the attitude towards recent advances in genetics, 72% had a neutral attitude, 16% a negative attitude and only 12% a positive attitude. Regarding the use of tests for choosing a partner for marriage, over 50% of respondents expressed an interest in genetic testing in the idea of having a deaf child "like me" or avoiding having a deaf child (Taneja et al., 2004). The precise goals of students who have expressed an interest in using genetic testing for partner selection and determining whether these attitudes will influence their behavior should be clarified.

The ethical debate regarding the right of the person to have a deaf child became more intense after the case of 2 lesbians who requested genetic tests to ensure that the future child will be deaf. To ensure that the future child will be deaf, a sperm donor was selected that was in the 5th generation of deaf (Mundy, 2002).

In the ensuing debate it was argued that this couple has the right to procreate what they want. (Savulescu, 2002). This led, in the deaf community, to a wide discussion about the implications of using genetic tests by the parents for deciding the born of a child with or without hearing loss.

Arguments from the Deaf community: These tests might negatively affect the child:

1. If you refuse to have a child (the child will no longer exist) - then it is worse than having a child who is deaf.
2. The deaf child is affected by this selection of being created only in the conditions in which his life will be so difficult that it does not deserve to be lived (Savulescu, 2002).

Arguments of the hearing people:

1. The right of the child to have a future with all possibilities.
   Davis Dena proposed an alternative analysis of this negative moral aspect of selecting for deafness: intentionally creating a deaf child is a moral injury because it dramatically affects the child's right to an open future (Davis, 2001).
2. The right of a child to have an open future is a term introduced by Joel Feinberg which refers to the collection of "anticipatory autonomy rights or rights-in-trust".

These "rights-in-trust" are rights that cannot be exercised by the child at that time, but are retained until the child becomes an adult. Although this right cannot be exercised by the child, it cannot be violated by the adult (Feinberg, 1980). This violation, Feinberg explains, guarantees that by the time the child becomes an adult, certain key options will already be closed for him. Example: a 6-year-old does not have the ability and the right to procreate but the ability to exercise this right in the future can be irretrievably compromised before reaching maturity. An adult sterilized in childhood will not be able to exercise the right to procreate (or choose not to), so sterilization is a violation of a child's right (Feinberg, 1980).

Deaf selection does the same thing by canceling a child's right because it isolates him / her from a community and also from a limited number of career opportunities. Davis Dena supports cochlear implantation for a child to develop multidimensionality in both communities, and at the age of maturity he can choose - the world of the deaf or the normal hearing (Davis, 2001). "The cochlear implanted deaf child is also a deaf child and he may generate deafness, but it is the moral duty of parents not to give them the right to an open future" (Davis, 2001).

These differences have significant social and cultural implications for the Deaf community causing distrust in opportunities offered by researchers. The Deaf community has two particularities in the ethics problem: there are no researcher from the Deaf community and there are no accessible information and research materials.

Collaboration with the Deaf community involves creativity, mutual respect, flexibility, compatibility, cultural competence and patience (McKee et al.,2013).

The deaf community is practically unstudied. Historically there was a eugenic movement (1880-1950) in the US, Anglia and Germany focused on eliminating deafness through medical technologies and genetic techniques. (Lane, 2005; Meador and Zazove, 2005; Padden and Humphries, 2006).

The moral permissibility of sterilizing the deaf was maintained in order to reduce "social burden" and increase the health of the human species through "better breeding".

Another movement called oralism prioritizes human speech over sign language (Lane, 2005; Greenwald, 2009).

The followers of "deaf culture" think that the last attack of eugenics and oralism consisted nowadays in the development of advanced medical techniques (cochlear implantation) that have led to a negative social perception of deafness as a disability rather than a cultural identity (Lane, 2005). Deafness as a cultural model must be preserved, but as a medical model it must be healed. Focusing on deafness as a disability (eg, the medical model of deafness) causes conflicting values and a sense of inferiority for deaf children and adults (Padden and Humphries, 2006; Hauser et al., 2010; McKee et al., 2013).

Gallaudet University - the most famous University for deaf people and university California - Los Angeles work with bicultural teams of normal hearing and deaf people on programs that not only identify deafness genes, but also allow researchers to explore the social impact of advanced genetic testing on deaf culture (Palmer et al., 2013).

In accordance with the ethical principle of beneficence, genetic research is focused on gaining scientific knowledge to improve the health of the deaf and not to eliminate them (McKee et al., 2013).

The right to have an open future, for as long as possible (an open future means not to limit or confine in any way the life endowment) does not apply to a child who is born with a disability. A deaf child with deaf parents can discover in his past the following: (1) parents knew that their child might be deaf but did not take either the hearing loss or the genetic screening and/or reproductive screening for diagnose and early treatment of deafness; (2) parents took the hearing loss screening but did nothing to rehabilitate their child; (3) parents used genetic techniques to be sure that their child will be deaf. The deaf child can accept the situation, or to claim sanctions or compensations from those he/ she considers to be responsible for his/her condition (parents, doctors), for his/her suffering and for his/her limited chances. This scenario is more and more possible.

Is it ethical to use genetic tests and reproductive techniques to decide the birth of a child according to his auditory status? The knowledge and techniques in the field of genetics have made rapid progress and the information provided by mass media has promoted the opportunities in this domain. Brugner (Brugner et al., 2000) in a study conducted in the USA in 2000, shows that 87% persons are willing to have genetic prenatal tests; Martinez in a study from 2003 indicates a percentage of 64% (Martinez et al., 2003), Middleton indicates that 28% of the deaf people are willing to have prenatal genetic tests (Middleton, 2004). The aim of genetic and reproductive techniques is to create an embryo without the genes of deafness or to detect the presence of such genes in the embryo.

In many cultures, societies or religions the use of such techniques is justified from a medical and ethical point of view. In some other areas, abortion is not allowed; in such cases for couples at risk, a solution could be the selection of germinal cells to create an embryo without disabilities. In this way, for those who do not accept abortion, the scenario implying avoiding deafness by genetic screening and reproductive techniques responds to the most important ethical criteria.

From the legal point of view, the molecular screening of hearing loss may be based on Article 34, 1st paragraph of the Constitution, which states that the “right to defend health is guaranteed” and in the 2nd paragraph where it is stipulated that “the state is obliged to take measures in order to ensure the population’s hygiene and health”. The molecular diagnosis in a child is possible only after having the agreement of the legal tutor and only if the screening is done in the benefit of the child or if the result of the test identifies the predisposition to a certain disease for the tested child or for his future siblings.

In the Additional Protocol of the Convention for Human Rights and Dignity of the Human Being, the genetic tests done for the sake of research in the field of biology and medicine are ruled out.

Conclusion

Genetic screening of deafness should be done in accordance with the objectives of the National Program. Its aims coincide with those of “The Principles of the Patient’s Rights in Europe”, which have been internalized through the adoption of the Law number 46 from 2003 regarding the patient’s rights, among which, it is recognized the right “for medical assistance of the highest quality”.

I.2. OPTIMIZING EARLY DIAGNOSIS OF DEAF CHILDREN

I.2.1 Optimization of audiological diagnosis of hearing loss in school children

Background

Neonatal hearing screening programs are expected to result in earlier intervention, it thus depends also on the healthcare process and organization after hearing screening (White, 2004). After early detection based on hearing neonatal screening, then objective diagnosis of the level of hearing loss and etiological diagnosis, the mode of rehabilitation depends on the level of hearing loss, associated symptoms and families' educative choices. To treat hearing loss, devices such as hearing aids or cochlear implants can be used the first one to amplify sounds and the second to replace the inner ear and to directly stimulate the cochlear nerve. When this is parents' choice, sign language and speech reading remain the last alternative for communication.

Cochlear implants restore useful hearing to profoundly or totally hearing-impaired patients. There is no comparable alternative medical treatment for profound-total deafness. Cochlear implants are electronic devices introduced surgically into the inner ear (i.e. class-III active medical devices). Unlike hearing aids, cochlear implantation necessitates a surgical procedure and incurs substantial costs throughout the lifetime of the recipient. In actual practice, the rehabilitation process has to be continued for several years, especially in children (Bouccara, 2005).

The deployment of cochlear implants has been spectacular in many countries for the last 20 years. The cochlear implant is the most successful neural prosthesis developed to date. More than 500,000 people had received a cochlear implant or bilateral cochlear implants in the beginning of 2018 (Smeds, 2018). This number exceeds by orders of magnitude the number for all other types of neural prostheses combined (Willson, 2011).

Like other medical devices, cochlear implants are highly regulated. European Directive 90/385 EEC relating to active implantable medical devices has introduced new rules according to the European Union competence to support, coordinate or supplement actions of the member states for better health protection (Art 168 TFEU). Cochlear implants need CE mark approval (through demand to a notified body, specified by national authorities in charge of the implementation of the directive) to be commercialized on the European market. As cochlear implant is an established technology and medical treatment with established safety and performance for more than 30 year' experience in hearing impaired children and adults, regulatory approvals is not considered a hurdle by companies, but a welcome aspect of market regulation to protect the users of all implant systems and ensure the standards are maintained by all concerned.

Due to the implementation of neonatal hearing screening it is now possible to identify in a reliable manner the existing hearing loss in the first months of life and to initiate the diagnostic steps to pinpoint the definitive diagnosis and to establish the appropriate treatment.

On the other hand, an auditory impairment acquired in pre-school and primary school children may remain unnoticed, being detected late when specific effects appear: vocabulary develops more slowly, the sentence structure is less complex, the speaking and diction are affected, the writing-reading processes can be delayed, the academic achievement are poor (Kenna, 2015).

In Auditory Neuropathy electrical signals transmitted from the cochlea to the brain are time distorted, so information is not relayed to the brain in a clear and consistent manner. The clinical manifestations of auditory neuropathy/dys-synchrony vary from near normal hearing to profound hearing loss in pure tone audiometry but with bad or no speech recognition (Starr et al., 1996; Kaga et al., 1996; Norrix and Velenovsky, 2014; Kaga, 2016).

Given the complexity of the evaluation of children with hearing loss or auditory processing problems, there is imperative necessary to use a comprehensive battery of appropriate and adapted audiological tests that are chosen based on the person's age, auditory problems, language and cognitive abilities.

The Romanian language particularities make impossible the translation and adaptation of speech tests from other languages due to the specificity of each language. Also, the frequency of each word in spoken language associated to language development is specific to each country. Every speech evaluation test battery should contain the most commonly used words and phonetically balanced material (Kral and O`Donoghue, 2010; Johns et al., 2012). Such an audiological test battery has not been created so far for Romanian language.

Personal contribution

Scientific and professional achievements:

Within this direction of the study concerning optimizing audiologic diagnosis of deaf children, the study performed by our team from Rehabilitation Hospital was the first Romanian audiological clinical test respecting all auditory test standards in terms of the homogeneity of the material. We have published this original research article in an ISI journal.

Published papers:
Cozma S, Dascalu C G, **Rădulescu L**, Martu C, Bitere O, Martu D, Olariu R. Audiological Clinical Validation of New Original Romanian Speech Audiometry Materials for Evaluation of Communication Abilities in Children of Primary School Age. Revista de cercetare si interventie sociala. 2016; (55):47-62. (IF =1.076)

Aim of the study

The aim of the study was to validate a speech audiometry test battery for Romanian language designed to be used in the evaluation of speech perception and speech production of children from primary school.

Material and methods

The study group included 24 children aged between 7 and 12 years old. The including criteria except the age were:

- healthy external and middle ear
- bilateral normal tonal audiometry
- age-appropriate cognitive and linguistic development
- good pronunciation
- Romanian native speakers.

All subjects in the study group were subjected to a full ORL examination:

- otomicroscopy
- tympanometry and,

- pure tone audiometry.

All audiometric assessment took place in a double-walled, sound-treated room in. The Interacoustics audiometer AD629 was calibrated according to up to date standards. For pure tone audiometry (PTA) and speech audiometry were used circumaural earphones (Sennheiser HDA 200). The Hughson-Westlake method was used for PTA procedure and the results were stored for further analyses in the database (Carhart and Jerger, 1959). Speech perception was tested monaurally in quiet using a recorded speech audiometry material.

Speech audiometry test battery was developed in our department. It consists of 50 phonetically balanced bi-syllabic words lists and 20 phonetically balanced monosyllabic words lists adapted to the level of language development for children from primary school. The linguistic material has been gathered based on the principles of early preschool education curriculum and primary school program developed and approved by Ministry of Education and Scientific Research. The most frequently used words according to school textbooks were incorporated in the test battery. The material was audio recorded in a professional studio by a female voice. The phonetically balanced words lists were generated using an original algorithm.

For every subject included in our study the pure tone average was determined in every ear using 3 frequencies (3 FAHL – Three Frequency Average Hearing Level: 500 Hz, 1000Hz and 2000 Hz) and also 4 frequencies (4 FAHL – Four Frequency Average Hearing Level: 500 Hz, 1000Hz, 2000 Hz and 4000 Hz). The lists were presented at different intensities to all children. For every list at a certain intensity was determined the Word Recognition Score (WRS - the percent of correctly recognized words) and for every tested ear were calculated the Speech Recognition Threshold (SRT - minimum hearing level for speech at which an individual can recognize 50% of the speech material) and the Maximum Recognition Threshold (MRT – minimum hearing level for speech at which the highest percentage of words are recognized).

The coding used for the generated lists was 7_12 – for the age range followed by B for bi-syllabic words lists and M for monosyllabic words lists and the list number with Arabic numerals (e.g. 7.B.1 for the first list of bi-syllabic words for a 7 years old child).

The speech audiometry test was performed in the same conditions as PTA and the results were stored in the database.

Statistical analysis of data was performed using software package SPSS 20.0.

Results

Descriptive analysis of the data indicated a relative homogenous group of study with 40% of boys and 60% of girls tested and having a balanced age distribution between 7 and 12 years with a mean of 9 years as shown in Fig. 1.a (SD = 1.384).

Eighty percent of children were tested with both bi-syllabic and monosyllabic words lists, while 16% have been tested only with bi-syllabic lists and 4% only with monosyllabic lists. The gender repartition of the group can be observed on the analysis of two categories of tested words lists (Fig. 1.b), where the girls are slightly better represented than boys; in the test room the girls were more cooperative in terms of test duration.

Word Recognition Score (WRS) for the 10 words lists for every tested intensity from 10 dB SPL to 100 dB SPL. For every intensity there are represented the mean of WRS with standard deviation intervals and the maximum variation. The figures 2 and 3 highlight this parameter for bi-syllabic and monosyllabic lists. It is easy to observe that the mean WRS riches over 95% at 30 dB SPL for both types of lists, starting with 40 dB SPL is constantly over 99% for all bigger intensities for bi-syllabic lists and over 98% for monosyllabic lists, suggesting that the speech recognition is a little more difficult for monosyllabic words, in agreement with all well-known principles of speech recognition tests.

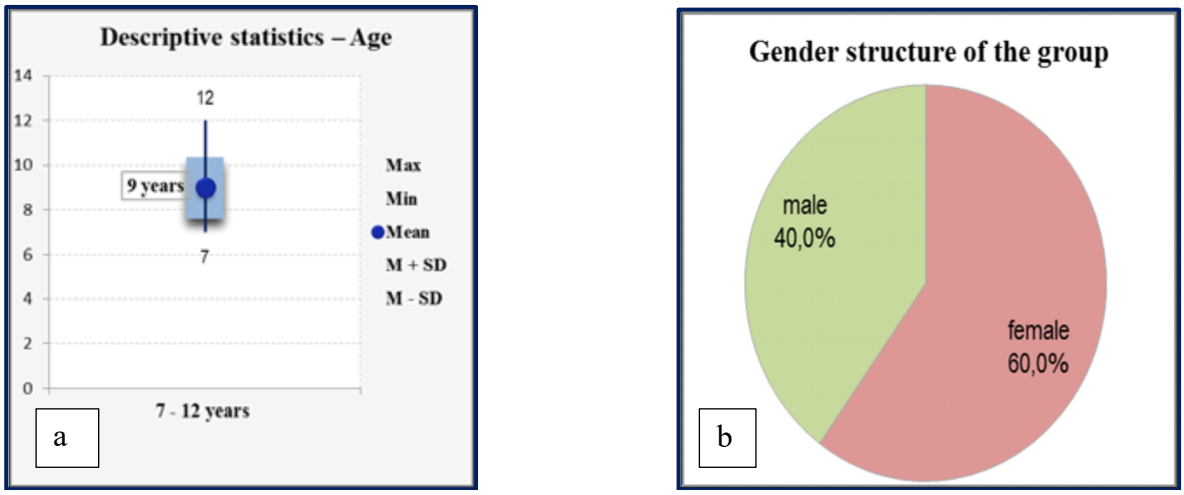


Figure 1.a and b - Age and gender distribution in the study group

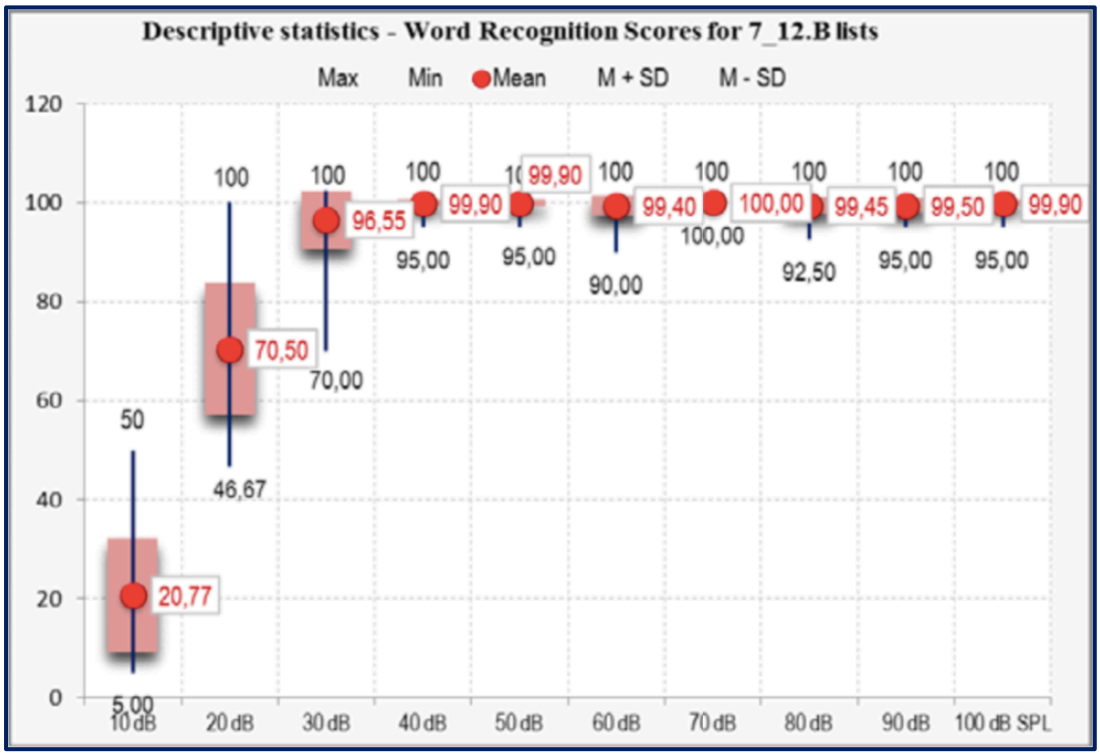


Fig. 2 Descriptive statistics – percentage of recognized words for disyllabic lists Note: Abscissa – intensities - dB SPL Ordinate – Word Recognition Score (WRS) %

For bi-syllabic lists presented at 30 dB SPL over 70% of children understood 100% of words (Fig. 4) and at 40 dB SPL over 97% present 100% intelligibility. As the level of presentation increases, we can note a tendency for decrease of intelligibility (less than 100%), but still rest for all subjects between 90 and 99%. Scores below 89% are shown only at intensity levels less than 30 dB SPL.

For monosyllabic lists (Fig. 5) the intensity level of presentation had to exceed 40 dB SPL to achieve the majority of subjects with 100% intelligibility (over 78%) and the rest between 90 and 99% (21%). We note two differences between monosyllabic lists: the maximum of speech performance is present at 30 dB SPL for bi-syllabic lists and at 40 dB SPL for monosyllabic lists and the intelligibility decreases slightly more for monosyllabic lists at 100 dB SPL intensity.

Disscusions

One of the most important and relevant audiological parameters is Word Recognition Score (WRS) for the 10 words lists for every tested intensity from 10 dB SPL to 100 dB SPL, with great importance for the 10 to 40 dB SPL in defining the shape of vocal audiogram.

The appropriate tests of speech perception in children should respect several criteria:

- cognitive, motor and, attentional demands of the test should be age-appropriate
- appropriate to a linguistically common level
- should assess a person's ability to communicate in everyday situations (Kosky and Boothroyd, 2003; Mendell, 2008).

Creating a language corpus for primary school children based on early preschool education curriculum and primary school program we reached the goal of word familiarity and age-appropriate linguistic level.

The speech audiometry doesn't test only the intelligibility of the spoken words, but also the speech production.

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The results of tested lists for each subject as well as for entire group for monosyllabic words and also for disyllabic words show parameters that fit with the normal vocal audiogram slope. The variability of the responses for normal hearing children for this group of age allows us to describe not just a regression line, but an area of the speech intelligibility audiogram that defines the normality (Fig. 2 and Fig. 3).

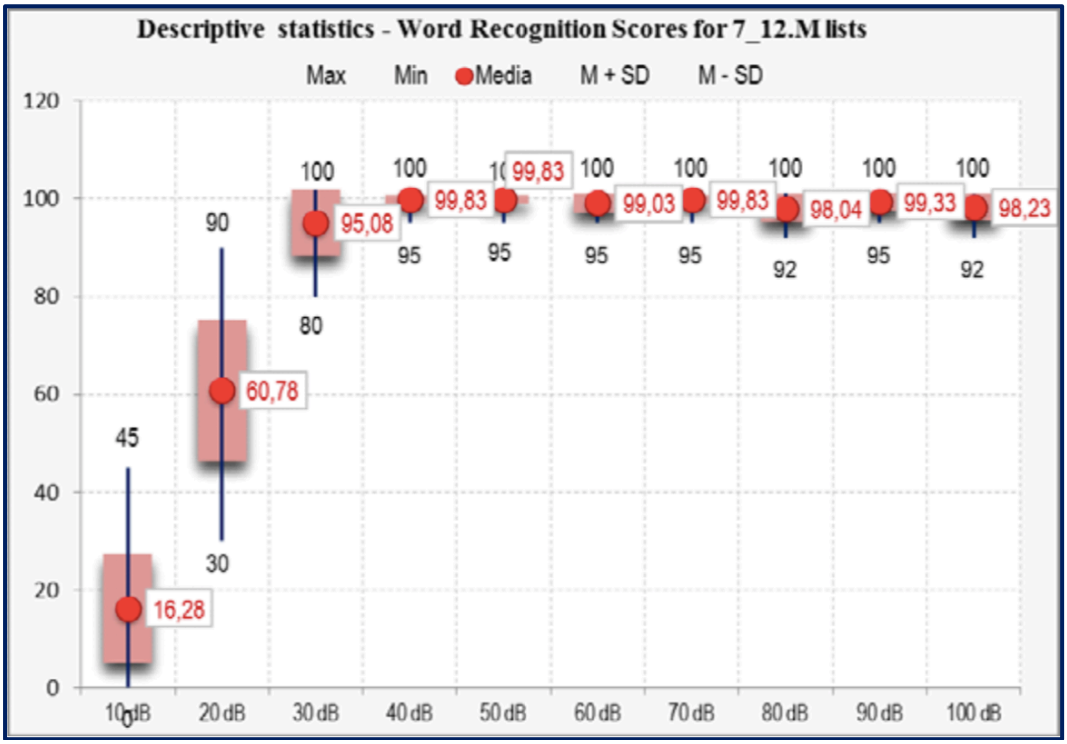

Fig. 3 Descriptive statistics – percentage of recognized words for monosyllabic lists

Note: Abscissa – intensities - dB SPL Ordinate – Word Recognition Score (WRS) - %

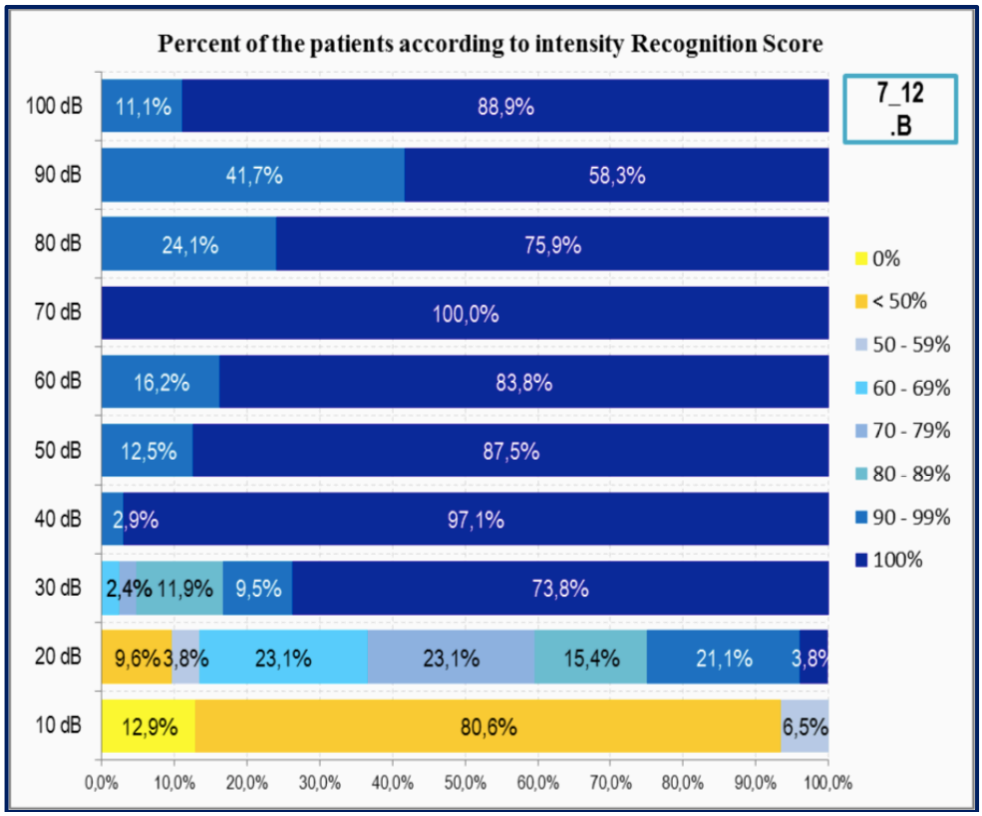

Fig. 4 Percent of the patients according to Word Recognition Score for all intensities in disyllabic words lists (7_12. B)

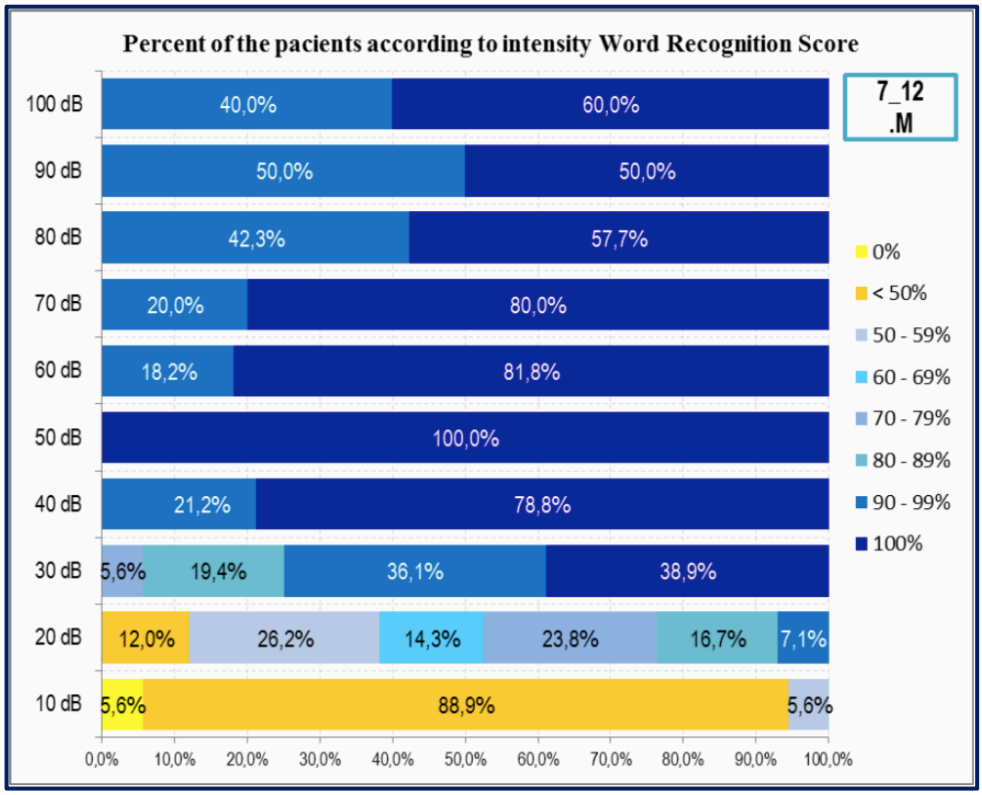

Fig. 5 Percent of the patients according to Word Recognition Score for all intensities in monosyllabic words lists (7_12.M)

We consider that this extended area including all the responses would define better, in a more accurate manner, the normal vocal hearing instead of a single curve results from the mathematical statistics.

In many studies referring to the modality of digitally recorded speech audiometry material developed for different languages, the audiological tests have been standardized with respect to audibility and psychometric function slope, being calculated for all words using logistic regression (Mullenix et al., 1989; Stach, 1995; Uhler, 2016). Usually the selected words from different languages were digitally adjusted, to create word recognition lists which are relatively homogeneous with respect to audibility and psychometric slope. The methodology of developing the SRT materials was by selecting a subset of disyllabic words with relatively steep psychometric function slopes and digitally equating their intensity to match the mean PTA of normally hearing subjects. We noticed in these studies the steepness of the shape of vocal audiogram, but as we mentioned before, we observed that a defined area of normal responses respects better than a regression line the variability of speech comprehension in the population of normal hearing children (Peterson and Lehiste, 1962; Spahr et al., 2014)

Any of audiometric slopes characterizing the speech recognition using these lists and which is projected in the defined area of normality can be considered within normal limits for speech intelligibility. We also observed that in the exposed methodologies for audiometry material development the focus was to create lists primarily according to the criteria of familiarity and auditory homogeneity among lists without attempting to phonemically balance the word lists. It is generally recognized the extreme difficulty, if not impossibility, to simulate the frequency of phoneme occurrence in a language with a list containing a limited number of words. (Harris et al., 2007; Nissen et al., 2007). We realized phonetically balanced words list using the most common words of spoken language.

Once validated for normal hearing for 7-12 years old, the tests can be applied in the audiological assessment of hearing threshold in children, to evaluate the speech intelligibility for hearing aided or cochlear implanted children. Speech perception assessment in children is essential in child's progress over time and the audiologic test battery that we created could determine the type of necessary interventions (amplification, speech therapy) for language and communications skills improvements.

The aim of the study was accomplished showing that the material we created in order to test the speech intelligibility for children aged between 7 and 12 years is a clinical valid tool. This is the first Romanian clinical validated test respecting all auditory test standards in terms of the homogeneity of the material: frequency of the words in the spoken language – communality factor; balanced frequencies spectrum representation similar to frequencies spectrum of common spoken language; known words corresponding to speech and cognitive specific age development, according to national preschool and primary school education curriculum.

Conclusion

The study demonstrates that any generated list with our algorithm can be clinically used with a high degree of confidence. Thus, the same results were obtained across multiple measurements with randomized different lists presentation, in similar standard testing conditions, without statistically significant differences between presented lists.

I.2.2 Optimization of etiological diagnosis of hearing loss in children

2.2.1 Prevalence of mutations at the DFNB1 locus

Background

Hearing loss (HL) is the most common sensory disorder in human, with an incidence at birth of 1 to 650 newborns (White, 2004; Smith et al., 2005) and a prevalence in the population of 10–12% (Mohr et al., 2000).

Due to its high prevalence, HL is placed amongst the major public health problems, hence the need of screening programs development (White, 2004; Joint Committee on Infant Hearing, 2007; Wood, 2013). The data collected from the universal newborn hearing screening, which we perform in our region, shows that out of the 7077 tested newborns in 2008 by otoacoustic emissions (OAE) – distortion products – 8 children were confirmed with bilateral severe or profound sensorineural hearing loss (SNHL), meaning 1.13 in 1000 newborns for our region.

The main purpose for early detection of children with HL is conventional hearing aid fitting or cochlear implantation at the right moment for optimal auditory and speech rehabilitation of the child (McPhillips, 2010).

As known, rehabilitation results are mainly related to the cochlear implantation age (Houston and Miyamoto, 2010) but also to other factors among which probably the HL etiology (Connell, 2007). HL can have a genetic cause, can be acquired or can be multifactorial (Parving, 1993).

Genetic deafness can be syndromic in 30% of cases (when it is associated with other pathologic findings) or non-syndromic in 70–80% of the total genetic deafness (Morzaria et al., 2004) – when it appears isolated to a seemingly normal person.

Genetic mutations responsible for HL cases can occur in autosomal or gonosomal chromosomes but also in mitochondrial DNA (Parving, 1993) et al.

The autosomal recessive forms represent about 80% of the total nonsyndromic deafness (Petersen and Willems, 2006) usually characterized by a sensorineural type involving the whole frequency range (Snoeckx et al., 2005). Most of these cases are severe forms with prelingual onset although some cases of post lingual onset with progressive evolution have also been described (Hochman et al., 2010). The DFNB1 locus on chromosome 13q11–12 is the most frequently affected one. Mutations located at this level are responsible for more than 50% of the autosomal recessive sensorineural hearing loss (ARSNHL) (Estivill et al., 1998). Two genes have been associated with the DFNB1 locus – GJB2 and GJB6 – involved in the auditory function by encoding the gap-junction proteins connexin 26 and connexin 30 (Kelley, 1999; Hilgert et al., 2009). Both connexins are components of the gap-junction channels that mediate electrolytic and other metabolites changes required for the inner ear function (Bruzzone et al., 1996).

In the GJB2 gene there were 91 described mutations, out of which 89 with recessive features. The prevalence of each mutation is different from one population to another according to the founder effect theory (Van Laer et al., 2001; Nance and Kearsey, 2004). In the Caucasian population the most frequent mutation is c.35delG (approximately 2/3 of cases) (Gasparini et al., 2000; Van Laer et al., 2001). In the Asian population the c.235delC mutation is the most encountered (Abe, 2000; Liu et al., 2002; Ohtsuka et al., 2003) while in the Jewish population the most frequent mutation is c.167delT (Morell et al., 1998). The p.R143W mutation is predominant among certain Africans (Kudo et al., 2000) and the p.W24* mutation among European Gypsy and Indian people (Ramshankar et al., 2003).

Mutations responsible for deafness can be monogenic (only one gene involved) or digenic (simultaneous involvement of 2 genes). Related to this, the role of GJB6 gene adjacent to GJB2 gene on the DFNB1 locus is described (del Castillo et al., 2005). The most frequent mutation in the GJB6 gene is a deletion – the 309-kb deletion (del Castillo et al., 2oo2). This deletion induces deafness either in homozygote form or in compound heterozygous form, in the last case only if there is a mutation at the level of the GJB2 gene on the pair chromosome. The prevalence of this deletion in the GJB6 gene is variable depending on the studied population (del Castillo et al., 2003).

Personal contribution

Scientific and professional achievements:

Within this direction of study concerning optimizing the etiological diagnosis of cochlear implanted children, in colaboration with "Albert-Ludwig" University from Freiburg we performed the first study that characterize and establish the prevalence of mutations at the level of the GJB2 gene, and to evaluate the prevalence of the deletions del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698) in the GJB6 gene in Eastern Romania. We have published an original research article, in a ISI journal. This study is of major importance in deciding Hearing Screening Protocol which entered in force this year.

Published paper:

Rădulescu Luminița, Mârţu C, Birkenhäger R, Cozma S, Ungureanu L, Laszig R
Prevalence of mutations located at the DFNB1 locus in a population of cochlear implanted children in eastern romania. Int J Pediatr Otorhinolaryngol. 2012; 76(1):90-94.

Aim of the study

All around the world significant efforts are being made to achieve the descriptive epidemiology related to connexin 26 mutations; in this regard, the main purpose of this study is to characterize and establish the prevalence of mutations at the level of the GJB2 gene, and to evaluate the prevalence of the deletions del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698) in the GJB6 gene in a population of cochlear implanted recipients in Eastern Romania, which makes it the first study of this type in our country.

The related objectives are: to contribute to the existing international database by gathering the information necessary to establish the implications of connexin 26 mutations in auditory and speech rehabilitation in cochlear implanted recipients and to gather useful data necessary for the genetic counseling and for the genetic screening of deafness.

Materials and methods

Patients - The study was performed on 45 patients from Eastern Romania, 25 males and 20 females (unrelated to each other) evaluated, diagnosed and cochlear implanted in the Department of Otorhinolaryngology, Head and Neck Surgery, Clinical Rehabilitation Hospital, University of Medicine and Pharmacy "Gr. T. Popa" in Iasi, Romania. The Ethics Committee of the University of Freiburg approved this part of the project (no. 161/02-07/2003/Birkenhäger). The study was also approved by the Ethics Committee of the Clinical Rehabilitation Hospital Iasi (no. 12511 – 10.07.2009).

The evaluation protocol consisted of: medical history of the disease, especially regarding the onset modality and evolution of deafness, associated symptoms and their development, past personal and family medical history to exclude any possible family interrelations and other possible causes of deafness, complete ENT examination – especially otomicroscopic exam, subjective and objective audiologic tests (pure tone and speech audiometry, OAE, auditory steady state response, tympanogram, auditory brainstem responses), vestibular testing, speech therapist examination, imaging testing – CT and MRI. Also, interdisciplinary complex examination (ophthalmologic, pediatric, clinical genetic, psychiatric, etc.) was performed to exclude syndromic forms.

All patients included in this study were cochlear implant users for congenital nonsyndromic or early onset idiopathic progressive severe (71–90 dB) to profound (>90 dB) SNHL. All of them have worn conventional hearing aids for at least 6 months – without any benefits – before being implanted. All were unilaterally implanted when included in the study. In cases with asymmetrical HL, the most impaired ear was implanted.

Molecular genetic analysis - Genomic DNA of patients was extracted from peripheral blood leukocytes of the patients using the standard methods (Qiagen, Hilden Germany). Primer and PCR conditions were selected according to procedures optimized previously for sequence analysis of exon 1 and the coding exon 2 of the GJB2 gene, including all splice sites (Birkenhaeger, 2006) and analysis of the GJB6 deletions del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698) (Wilch, 2010). Sequencing of the PCR products was done with standard procedures and analyzed in an automated DNA sequencer Amersham MegaBACE™ 500 (Amersham Biosciences, GE Healthcare Europe, München, Germany).

For the examination of the GJB6 gene (connexin 30) deletions the breakpoint junctions of del(GJB6-D13S1830) and del(GJB6-D13S1854) were analyzed according to del Castillo et al. (2003, 2005) (del Castillo, 2003; del Castillo, 2005) the deletion del(chr13:19,837,343–19,968,698) was analyzed according to Wilch (Wilch et al., 2010).

Informed consent was obtained from the patients, parents or legal guardians for children before collecting blood for genetic testing.

Results

Our study included 45 cochlear implanted patients, 43 had bilateral congenital severe to profound SNHL and 2 had progressive HL that required cochlear implantation at ages of 6 and 8 years, respectively. The genetic analysis of the GJB2 (connexin 26) and GJB6 (connexin 30) genes identified in 22 (48.8%) patients mutations in the GJB2 gene: 12 (26.6%) patients were homozygous for the c.35delG mutation; 5 (11.1%) patients were compound heterozygous with c.35delG mutation on one allele and a different mutation on the other allele; 1 (2.2%) patient was compound heterozygous for two different mutations non c.35delG; in 4 (8.8%) patients only one mutated allele was identified (Table V).

The analysis of 90 alleles revealed 6 different mutations, four of these alterations are truncating mutations, c.35delG, c.71G>A p.W24*, c.299_300delAT and c.313_326del14 (AAGTTCATCAAGGG), the two other alterations are non-truncating mutations, c.358_360delGAG p.delE120 is a in frame deletion and c.551G>C p.R184P is a missense mutation. The truncating mutation c.35delG represents 80% of all the mutated GJB2 (connexin 26) alleles, the prevalence of this mutation is 35.5% (32/90) in the investigated collective of patients all the mutations p.W24*, p.R184P and c.313_326del14 have a prevalence of 2.2% (2/90) and the mutations c.299_300delAT, and p.del E120 have a prevalence of 1.1% (1/90) (Table VI).

Table V - GJB2 and GJB6 genotypes

GJB2 and *GJB6* genotypes found in 22 subjects out of a collective of 45 patients with non-syndromic hearing loss.

Compound homozygous	
c.35delG/c.35delG	12
Compound heterozygous	
c.35delG/c.313_326del14	2
c.35delG/c.71G>A	1
c.35delG/c.551G>C	2
c.71G>A/c.299_300delAT	1
Heterozygous affected	
wt/c.35delG	3
wt/c.358_360delGAG	1
Total mutated subjects	22
No mutation found	23
Total subjects	45

The GJB6 (connexin 30) deletions GJB6-D13S1830, GJB6-D13S1854 and del(chr13:19,837,343–19,968,698) were not detected in our patients (Lerer et al., 2001; del Castillo et al., 2002; del Castillo et al., 2005; Wilch et al., 2010).

Additional no polymorphisms were identified.

Additional exon 1 was analyzed in all individuals who proved to be heterozygous for only one coding mutation, to identify the splice-site mutation IVS1+1G>A.

Both patients with progressive SNHL were compound heterozygous (c.35delG + c.313_326del14 and c.35delG + c.71G>A p.W24*).

Discussions

The present study, like many others published in the recent years, describes the genetic mutations present on the DFNB1 locus in cochlear implanted recipients. Although, significant efforts have been made worldwide to define the epidemiology of GJB2 and GJB6 genes mutations related to HL, the issue remains open for some populations.

This is the first report in Romania of the genetic profile of a group of cochlear implanted recipients with bilateral severe to profound SNHL due to mutations in the GJB2 and GJB6 genes.

A previous study, performed in the Northwest region of Romania, determined only the prevalence of two mutations (c.35delG and p.W24*) in the GJB2 gene, in non-cochlear implanted patients with different degrees of SNHL (Birkenhaeger et al., 2006; Lazar et al, 2010).

As our study enrolled only patients with idiopathic deafness, a large number of cases (40%) with genetic HL secondary to DFNB1 mutations were identified, which is consistent with the literature data according to which the GJB2 mutations are the most frequent cause of ARSNHL in most world populations, accounting for up to 50% of ARSNHL cases (Snoeckx et al., 2005) These findings emphasize the importance of GJB2 screening in our population for early detection of severe to profound hearing loss.

The c.35delG mutation is most frequently occurring in the Caucasian populations and may account for up to 70% of all GJB2 mutations. As we had expected the most frequent mutation in our group was c.35delG in homozygote state present in 66.7% of the patients with GJB2 mutations.

Table VI Spectrum of GJB2 (Connexin 26) mutations detected in non-syndromic hearing loss in our group.

Spectrum of *GJB2* (Connexin 26) mutations detected in non-syndromic hearing loss in our group.

Nucleotide	Protein	Classification	Mutation type	No.	References
c.35delG	p.Gly12Valfs*2	T	Deletion/Nonsense	32	Denoyelle et al. (1997) [48]
c.71G>A.	p.Trp24* (p.W24*)	T	Nonsense	2	Kelsell et al. (1997) [49]
c.299_300delAT		T	Frameshift	1	Abe et al. (2000) [26]
c.313_326del14	p.Lys105Glyfs*5	T	Deletion/Frameshift	2	Denoyelle et al. (1999) [50]
c.358_360delGAG	p.delGlu120 (del E120)	NT	In Frame Deletion	1	Denoyelle et al. (1999) [50]
c.551G>C	p.Arg184Pro (p.R184P)	NT	Missense	2	Denoyelle et al. (1997) [48]
T, Truncating mutation; NT, non-truncating mutation			Total mutated alleles:	40	

In the last years, **after the publication of our study**, two more recessive mutations at the DFNB1 locus have been reported:

- one of them includes both *GJB2* and *GJB6* genes (Feldmann et al., 2009).
- the other is a deletion that removes a 131-kb fragment in the DFNB1 region without affecting either *GJB2* or *GJB6* (Wilch et al., 2006), which might be a regulatory element necessary for the expression of CX26 and/or CX30 in the inner ear.

The high number of mutations identified in the GJB2 gene (6 different mutations in 22 of the 45 tested patients – Table V) in our patients has at least two implications:

(1) It points out the high prevalence of GJB2 mutations in cochlear implanted children from our region (when compared with studies that had found low incidence of GJB2 mutations in certain populations with severe to profound SNHL) demonstrating the importance of GJB2 analysis in our population.

(2) The presence of 5 mutations, different from c.35delG, in 33.3% of cases emphasizes the importance of genetic analysis by direct sequencing of the entire GJB2 gene rather than only by genetic testing common mutations exclusively (Fitzgerald, 2004); according to the connexin-deafness homepage, 91 different mutations have been identified, some of which are very frequent and others are extremely rare. These mutations occur with different frequency across populations. As the mutations reported after 2003 are not listed, an extensive literature search completed by Hilgert et al. estimates that over 220 mutations have been reported. In the patients here under analysis no new mutations had been observed (Hilgert et al., 2009).

The second most common mutation (p.W24*) was found in compound heterozygous form in 2 of 18 patients with connexin 26 mutation on both alleles (Table VI). This mutation is considered characteristic for the European Gypsy and Indian Population (Ramshankar et al., 2003). These findings are similar with those reported by other authors for the Central and Eastern Europe (Freiet al., 2002; Uyguner et al., 2003, Minarik et al., 2003; del Castillo et al, 2005).

Four (8.9%) heterozygous patients were found, 3 (6.66%) for the c.35delG mutation and 1 (2.22%) for the c.358_360delGAG without any other alteration either on the GJB2 or GJB6 gene on the second allele. This cannot explain the hearing impairment of these patients [25]. The etiology of deafness in these patients is possible to be secondary either to a nongenetic factor or to another mutation unrelated to DFNB1 locus and the affected patients can be carriers of GJB2 mutations.

The fact that none of the patients included in the study had the GJB6 gene deletions, that we analyzed, implies that further studies on greater populational groups are necessary to evaluate the epidemiologic significance of these mutations in our country.

The deletion del(GJB6-D13S1830), del(GJB6-D13S1854) and del(chr13:19,837,343–19,968,698), specific to West Europe, is otherwise rare or even absent in Eastern Europe countries (rare in Czech population (Fitzgerald et al., 2004; Seeman et al., 2005), absent in Austria (Wilch et al., 2010; Frei et al, 2004). and Croatia (Toth et al., 2004; Medica et al., 2005).

The findings that the number of deaf persons carrying a single GJB2 mutation is higher than expected led to a search for other mutations in, or near GJB2. As a result there have been identified two large deletions: del(GJB6-D13S1830), del(GJB6-D13S1854) and the deletion del(chr13:19,837,343–19,968,698) (Wilch et al., 2010). These deletions truncate the neighboring GJB6 gene and inhibit the GJB2 gene expression (by deleting probably a GJB2 regulatory element not yet identified) so that they may be considered GJB2 mutations as well. Literature data shows that these mutations are usually found in compound heterozygosity with a GJB2 coding mutation causing significantly worse HL than most other GJB2 mutations (del

Castillo et al., 2005). One argument for this might be that the expression of both copies of GJB2 and one copy of GJB6 is abolished. The del(GJB6-D13S1830) deletion seems to be worldwide spread with a much higher occurrence rate than del(GJB6-D13S1854) – found mainly in Spain and the UK (Pallares-Ruiz et al., 2002; del Castillo et al., 2005). There are significant more GJB2 mutation carriers without any of these 2 deletions, indicating that other unidentified mutations/deletions may be present at the DFNB1 locus, or that another HL cause may be involved.

As to the genotype–phenotype correlation, mention should be made of the fact that even if the literature states that HL caused by connexin 26 mutations is non evolutive (Snoeckx et al., 2005) – our group includes 2 patients with progressive idiopathic deafness, with early-onset. Progressive hearing deterioration, up to the point where conventional hearing aids become useless (at ages of 6 and respectively 8 years in our group), requires cochlear implantation. The genetic profile of these patients was compound heterozygous in both cases (c.35delG + c.313_326del14 and c.35delG + c.71G>A p.W24*). However, nowadays, more and more authors report cases with SNHL with early-onset and progressive evolution in patients with connexin 26 mutations (Hochman et al., 2010; Pagarkar et al., 2006).

In 2014 Chen shows that congenital deafness induced by Cx26 deficiency is associated with cochlear developmental disorders rather than hair cell loss and EP reduction (Chen and Oghalai, 2016)

More recently, a study published in 2015 found that after deletion of Cx26 in the cochlea of experimental animal the hearing remains normal at young ages. The authors assume that Cx26 deficiency may not impair K^+-recycling and Ca^{++}-wave propagation in the cochlea or that impaired K^+-recycling and Ca^{++}-wave propagation do not lead to hearing loss (if Cx26 deficiency can in fact impair K^+-recycling and Ca^{++}-wave propagation in the cochlea). In the same study, the authors found that Cx26 deficiency induced late-onset hearing loss may result from the reduction of active cochlear amplification rather than possible impairment in K^+-recycling and Ca^{++}-wave propagation (Prera et al, 2014; Zhu et al., 2015).

No possible correlation could be established between deafness degree and genotype, because the studied group consists only of patients with severe to profound SNHL that were cochlear implanted.

At individual level, knowing the genetic etiology is essential for the genetic counseling. There is a 25% recurrence chance for parents that have one child with GJB2-related deafness to have another child with the same genotype. Also, there is a 66% chance that the second child will have mild-to-moderate HL and a 34% chance that the HL will be more severe if their first child has mild-to-moderate HL. Furthermore, progress made in characterization of genotype–phenotype correlations allows appropriate informing regarding prognostic implications. Several studies have shown that in such a child with severe-to-profound HL that receives a cochlear implant, the parents can expect their child to have an excellent outcome (Connell et al., 2007). A genetic diagnosis can be beneficial to parents preventing sometimes parental guilt and anxiety (Denoyelle et al., 1999; Connell et al., 2007). This creates the opportunity to make accurate genetic advice possible and provides prognostic information. Beside the fact that genetic testing has a very important role, test results must be thoroughly interpreted, and efforts must be taken to ensure that parents and family members understand the information presented to them. A great deal of attention is needed to take responsibility for these matters.

At a more general level, the knowledge of mutations prevalence in a certain population allows the development of adjusted screening programs.

Conclusions

The genetic analysis of GJB2 mutations revealed that 48.8% of cochlear implanted patients present mutations of connexin 26. We found an important number of different

mutations (6 different mutations) with implications in hearing screening programs development in eastern Romania. The most prevalent mutation of GJB2 gene was c.35delG mutation. We did not find any new mutation. The connexin 30 studied deletions were not detected in our group.

2.2.2 Molecular analysis of rare non-syndromic hearing loss

Background

According to OMIM, approximately 1738 gene–disease relationships were discovered between 2010 and 2016 (Boycott et al., 2017)

The auditory system is highly complex, and genetic hearing loss is highly heterogeneous.6 There are over 150 genes proposed to be associated with nonsyndromic hearing loss (NSHL) and over 400 genes associated with syndromic forms of hearing loss (Hereditary Hearing Loss Homepage; http:// hereditaryhearingloss.org; Abou Tayoun et al., 2016).

To date more than 6,000 mutations in more than 150 genes have been causally implicated in deafness (Sloan-Heggen et al., 2016; Oza et al., 2018).

The goal of Clin Gen Hearing Loss Clinical Domain Working Group (Di Stefano et al., 2019) was to evaluate the relation between different reported mutations and the presence of deafness. In a paper published in march 2019 the group performs evidence-based curation of 142 genes associated with non-syndromic and syndromic hearing loss, consisting of 164 gene–disease pairs with 82 Definitive (50%), 12 Strong (7%), 25 Moderate (15%), 32 Limited (20%), 10 Disputed (6%), and 3 Refuted (2%) (https://search.clinicalgenome.org/kb/gene-validity).

After 2011 we have continued the genetic analysis of DFN B1 locus in congenital deaf implanted children in our department. The sequence analysis of the gene GJB2 (Connexin 26) and the deletion analysis of the gene GJB6 (Connexin 30) was performed so far in 231 cochlear implanted children. In these patents we identified different mutations in the gene GJB2 (Connexin 26) in 122 patients represented 50% from all cochlear implanted patients that were tested, and no deletion in the gene GJB6. Otherwise, GJB6 was one of the analyzed genes by the Clin Gen Hearing Loss Clinical Domain Working Group. The panel concluded that the relations between autosomal recessive non syndromic hearing loss and GJB6 mutations were present just when large deletions, including GJB6-D13S1830 and GJB6- D18S1854 were documented. These deletions were shown to abolish expression of the cis-GJB2 allele. Also, it was confirmed in mice that regulatory region 5′ of GJB6, but not the gene itself, is necessary for normal hearing. The panel decided that coding variants in GJB6 are not associated with hearing loss (DiStefano et al., 2019).

In 2019 we began to look for other mutations in the 109 children who were free of mutations in GJB2 and GJB 6 genes.

Personal contribution

Scientific and professional achievements:

In colaboration with ”Albert-Ludwig” University from Freiburg we continued the study concerning optimizing the etiological diagnosis of cochlear implanted children in the attempt to discover possible mutations in some other genes responsible of deafness in cochlear implanted children in Eastern Romania. We have published our results in an original research study as a poster in a ISI journal.

Published paper:

Trabandt M, **Rădulescu L**, Laszig R, Birkenhäger R. Molecular genetic analysis of rare non-syndromal prelingual hearing disorders in a Romanian patient collective Laryngo-Rhino-Otol 2019; 98(S 02): S330 DOI: 10.1055/s-0039-1686527

Aim of the study

The aim of the study was to clarify which other genes were potentially involved in prelingual severe to profound hearing impairment in the group of cochlear implanted children.

Material and methods

One hundred nine patients have been included in our study, they have been diagnosed with severe non-syndromic hearing impairment in their first two years of life and who have been shown to have no changes in the DFNB1 gene locus or GJB2 and GJB6 gene, respectively. The detection of genetic alterations was carried out by bi-directional sequencing of the coding exons, as well as the intron transitions.

Results

First of all, the genes GRXCR1 and ESRRB were analyzed in this patient group followed by genes TMIE, GIPC, CLDN14, CABP2 and LHFPL5. By DNA sequencing, 2 mutations, 5 unknown polymorphisms and 10 known alterations that are already cataloged in the databases of international sequencing projects have been detected so far (Table VII).

Conclusions

In the investigated patients, mutations and previously unknown polymorphisms were occasionally characterized in the genes GRXCR1, ESRRB as well as TMIE, GIPC and LHFPL5, however, an accumulation of changes is not available, therefore, further investigations are required to be able to better characterize the etiology of prelingual hearing disorders.

Table VII Identified polymorphisms and functionally relevant mutations in genes for prelingual non-syndromic autosomal recessive inherited hearing disorders.

Gen	SNP`s	heterozygote Mutationen	homozygote Mutationen
TMIE	4	1	1
GIPC3	2	3	1
CLDN14	6	1	-
LHFPL5	-	2	-
GRXCR1	4	2	2
ESRRB	5	3	-

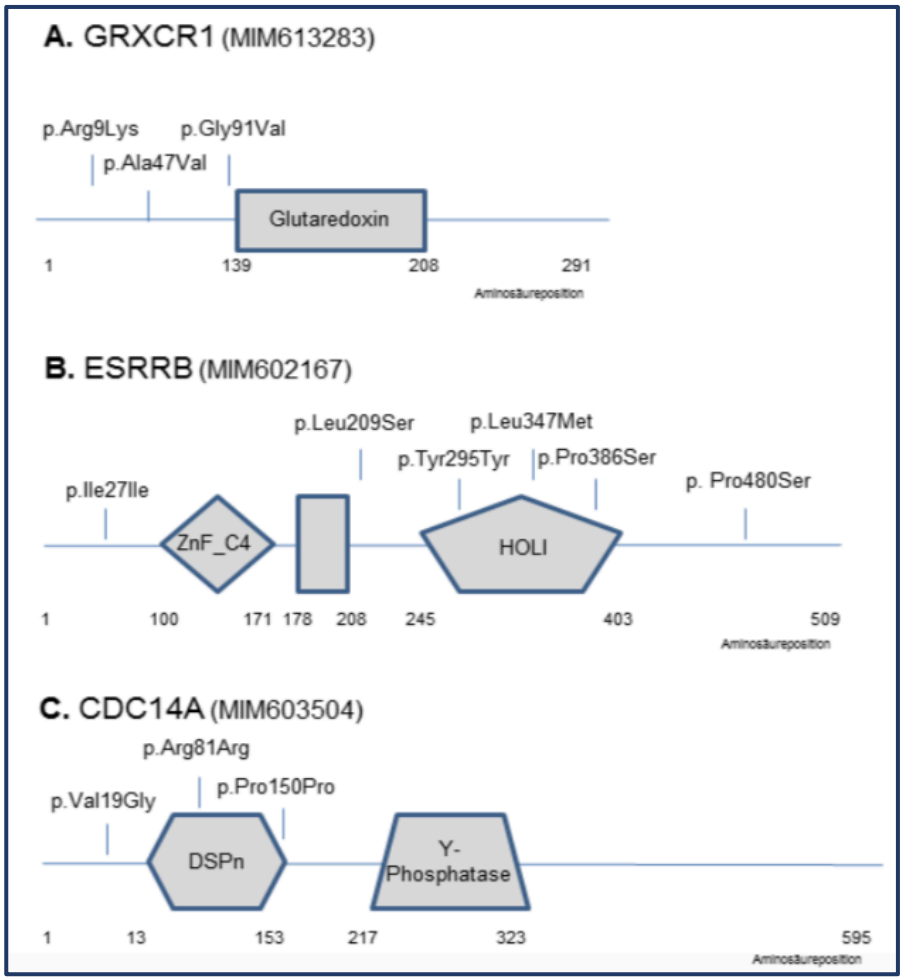

Fig. 6 Mutation analysis of the genes GRXCR1, ESRRB and CDC14A, secondary structure of the gene domain functional products, glutaredoxin (disulphide reductase), ZnF_C4 (transcriptional regulator, hormone binding domain), HOLI (ligand-binding domain of hormone binding domain). Receptors DSPn (subunit – protein phosphatase) and Y-phosphatase, as well as the position of previously identified mutations and polymorphisms.

D. Protein sequence alignment

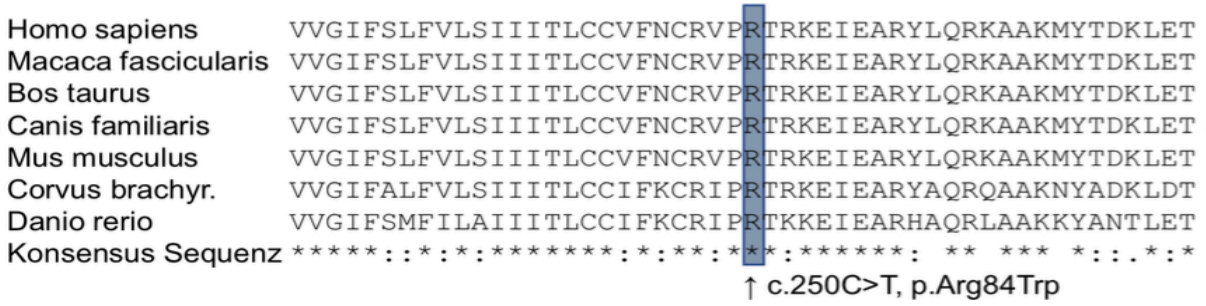

Fig. 7 Mutation analysis using the example of the identified changes in the TMIE gene (A)

Position of the amino acid exchanges in the protein domains. (B) Sequence alignment between different species reveals that the p.Arg84Trp mutation affects a highly conserved amino acid. (D) electrogram of the homozygous mutation pArg84Trp; (C) control sequence.

I.3. OPTIMIZATION OF TREATMENT OF DEAF CHILDREN

I.3.1 Rational of corticotherapy in sudden deafness

Background

The annual incidence 5-30 cases per 100 000 persons, 15 000 cases annually reported worldwide and 4 000 cases annually reported in USA (Alexander and Harris, 2013). The incidence of sudden sensorineural hearing loss (SSNHL) in pediatric patients is unknown (Tarshishet al, 2013). Response to steroids varied from complete resolution of SSNHL to worsening.

Hearing improvement occurred in 50% of children treated with oral steroids. Intratympanic steroid treatment is another option but may have practical limitation in the pediatric population (Dedhia et al., 2016).

Sudden sensorineural hearing loss is an emergency in otolaryngology. It has been defined as 30 dB or more sensorineural hearing loss over at least three contiguous audiometric frequencies occurring within 3 days or less. The specific cause is identified in about 10-15% of cases (Cavaleriu et al., 2013). Various infective (especially viral) (Stokroos et al., 1998), vascular, and immune causes (Cogan's syndrome) (Berrocal and Ramirez-Camacho, 2002) have been proposed, with vascular disorders in cochlear terminal vascularization playing an important role (Trune and Nguyen-Huynh, 2002).

There are ototoxic drugs that can damage hearing, such as antibiotics (aminoglycosides), diuretics and certain anticancer drugs. Acoustic trauma or trauma such as head injuries and temporal bone fractures can cause SSNHL. About 10% of people with Meniere's disease experience SSNHL. In addition, tumors such a vestibular schwannoma or cerebellopontine angle (CPA) tumors can cause SSNHL. There are many potential causes of SSNHL, but despite extensive evaluation, the majority of cases remain idiopathic.

An increase in reactive oxygen species (ROS) production is assumed to play an important role in sudden hearing loss (Halliwell et al, 1992). Recent studies have shown the importance of oxidative stress, defined as an excess of pro-oxidant species not counterbalanced by an adequate endogenous and exogenous antioxidant defense system (Campise et al., 2003; Sachdev and Davies, 2008), as a risk factor for microvascular damage in different vascular disorders (Halliwell and Gutteridge, 1984; Son, 2007).

The most common ROS are superoxide anion (O_2^-), hydroxyl radical (OH^-), hypochlorite (OCl^-) and nitric oxide (NO^-). ROS are converted to nonreactive molecules by endogenous cellular enzymes, such as copper/zinc superoxide dismutase (SOD1), manganese superoxide dismutase (SOD2), catalase and peroxidase. They are generated in vivo as a byproduct of mitochondrial respiration and are also produced via autooxidation of chemical and biological molecules (Seidman et al., 1997).

The imbalance between ROS and total antioxidant capacity (TAC) is thought to be a potential pathogenetic mechanism leading to endothelial dysfunction. ROS derived from various oxidation pathways can generate products leading to cellular deregulation (Capaccio et al, 2012). ROS directly damages subcellular structures, such as mitochondrial DNA (mtDNA), creating deletions and mutations, lip id peroxidation, polysaccharide depolymerization, nucleic acid disruption and oxidation of sulfhydryl groups, leading to enzyme inactivation and subsequently producing bioenergetically deficient cells. This cascade of events is responsible

for the reduction in the mitochondrial membrane potential and the loss of cochlear hair cells, with an attendant increase in the auditory threshold leads to hearing loss (Darrat et al., 2007).

Regarding the treatment of sudden hearing loss, an important number of clinical studies have noted that the best results in terms of hearing recovery were obtained after corticosteroid administration. Glucocorticoids have been widely used as a therapeutic drug for sudden sensorineural hearing loss (Wilson et al., 1980). However, very little is known about the mechanism(s) underlying the protective effect of glucocorticoids against hearing loss (Nagashima and Ogita, 2006).

Personal contribution

Scientific and professional achievements in this field :

Within this clinical study implying a new direction in the treatment of Sudden deafness it is evaluated the effect of steroids in reducing the oxidative stress in the cochlea. We found that the antioxidants enzyme (SOD and GPX) activities increases and the concentration of MDA decreases after systemic corticoid treatment. We have published one original research article, in a ISI journal.

Published paper:
Cavaleriu B, Mârţu D, Hrițcu I, Manolache O, **Rădulescu L.** Idiopathic sudden hearing loss: oxidative status before and after corticoid treatment. Archives Biological Science Belgrade. 2015; 67(4):1297-1302.

Aim of the study

The aim of the study was to evaluate the effects of steroid treatment on superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities and malondialdehyde (MDA) concentration by measuring blood serum-level changes after steroid therapy in sudden sensorineural hearing loss.

Materials and methods

This study included 15 patients with sudden sensorineural hearing loss, according to American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) guidelines of idiopathic sudden sensorineural hearing loss (ISSNHL) and were enrolled at the Otolaryngology Clinic of the Recuperare Hospital between September 2013 and July 2014.

Inclusion criteria comprised patients with sudden sensorineural hearing loss of more than 30 dB over at least three contiguous frequencies with pure tone audiometry observed within 72 h of onset. Exclusion criteria included other etiologies than idiopathic hearing loss (e.g. autoimmune diseases, history of cardiovascular disease or renal insufficiency and diabetes mellitus, head trauma with rupture of round windows membrane, and tumoral cerebral mass −vestibular schwannoma or cerebellopontine angle tumors).

This study was conducted according to the provisions of the Helsinki Declaration and all patients signed a written consent for participation in this study. A careful history and detailed medical examination were made with special attention directed toward the onset time, possible causes and associated symptoms.

The patients underwent intravenous systemic administration of 250mg/day of the corticosteroid, methylprednisolone sodium succinate (Solu-Medrol$^{(r)}$, Pharmacia Enterprises given for 7 days, with audiological and clinical follow-up at the end of the therapy, and one month post-event.

Sample collection and laboratory methods

Blood samples were drawn in the morning from each patient before corticoid treatment and 12 h after the last corticoid administration and were collected in light-protected tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Serum aliquots were frozen after separation and stored at -80°C until assayed. After treatment, only ten patients accepted blood sampling.

Determination of SOD activity

The activity of superoxide dismutase (SOD) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 mL reaction mixture contained 100 mM TRIS/HCl (pH 7.8), 75 mM NBT, 2 µM riboflavin, 6 mM EDTA, and 200 µL of blood serum. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as previously described by Winterbourn et al. (1975). The enzyme activity is expressed as units/mg protein.

Determination of GPX activity

Glutathione peroxidase (GPX) activity was analyzed by spectrophotometric assay. A reaction mixture consisting of 1 ml of 0.4 M phosphate buffer (pH 7.0) containing 0.4 mM EDTA, 1 mL of 5mM NaN_3, 1 mL of 4 mM glutathione (GSH), and 200 µL of blood serum was pre- incubated at 37°C for 5 min. Then 1 mL of 4 mM H_2O_2 was added and incubated at 37°C for a further 5 min. The excess amount of GSH was quantified by the DTNB method as previously described by Sharma and Gupta (2002). One unit of GPX is defined as the amount of enzyme required to oxidize 1 nmol GSH/min. The enzyme activity is expressed as units/mg protein.

Determination of MDA level

Malondialdehyde (MDA), which is an indicator of lipid peroxidation, was spectrophotometrically measured using the thiobarbituric acid assay as previously described by Ohkawa et al. (1979). Two hundred µL of blood serum was added and briefly mixed with 1 mL of 50% trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95°C for 20 min. Samples were centrifuged at 960 x g for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein.

Estimation of protein concentration

Estimation of protein was done using a BCA protein assay kit (Sigma-Aldrich, Germany). The BCA protein assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein, as previously described by Smith and colleagues (Smith et al.,1985).

Statistical analysis: Student t-test for paired data. P values less than 0.05 were considered statistically significant.

Results

Experimental data were registered after 7 days of corticoid systemic administration. The corticoid treatment significantly enhanced the SOD ($p<0.002$) (Fig. 8) and GPX activities ($p<0.0001$) (Fig. 9) compared to initial levels (before corticoid treatments).

Oxidative stress generates free radicals that induced peroxidation of the membrane lipids resulting in the formation of MDA. The corticoid treatment significantly decreased the MDA concentration ($p<0.0003$) (Fig. 10).

Disscusions

Steroid treatment significantly increased the endogenous antioxidant status in SOD and

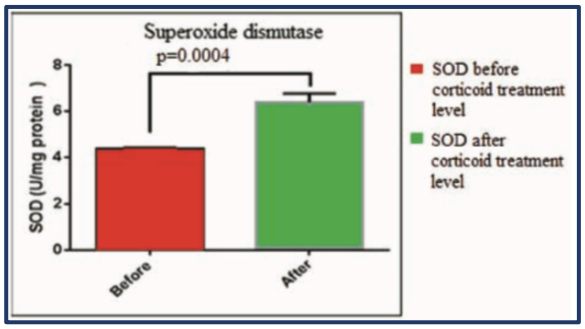

Fig.8 Effect of corticoid treatment on SOD activity. Data are presented as the mean ± SEM; *p<0.05 vs. initial concentration

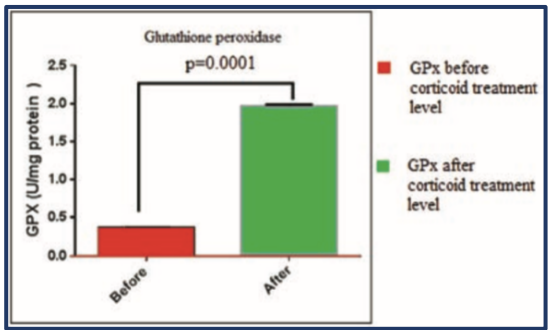

Fig. 9 Effect of corticoid treatment on GPX activity. Data are presented as the mean ± SEM; *p<0.05 vs. initial concentration

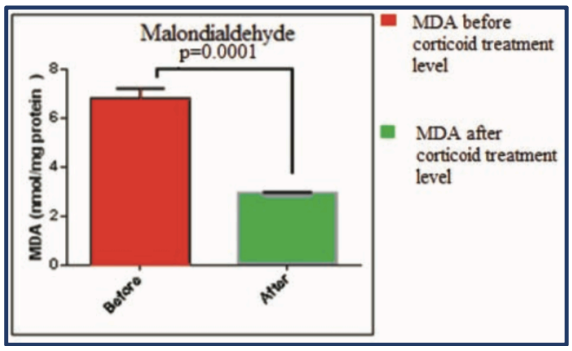

Fig. 10 Effect of corticoid on MDA activity. Data are presented as the mean ± SEM; *p<0.0001 vs. initial concentration.

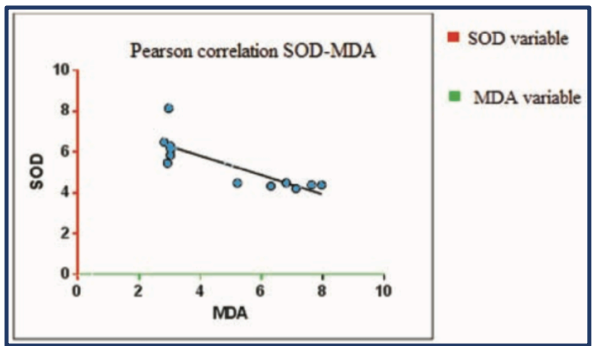

Fig. 11 Pearson correlation SOD-MDA.

GPX activities. A decrease in the activities of these free radical scavenging enzymes could result in the generation of superoxide anions and hydrogen peroxide, which in turn produce hydroxyl free radicals, the cause of many toxic reactions.

When linear regression was calculated using post-corticoid treatment values, a significant correlation between SOD and MDA (r = -0.8214) (Fig. 11) and GPX vs. MDA (r = 0.9977) (Fig. 12) was observed. These results suggest that of the increase in antioxidant defense was related to a significant decrease in the lipid peroxidation (MDA) level.

Total hearing recovery was observed in 3 patients, partial recovery in 7 and no recovery in 5 (Fig.13). A statistically significant difference was found between initial audiometry values and after corticoid treatment, p = 0.0002 (p<0.005) for the recovery group. No side effects or complications were noted following treatment.

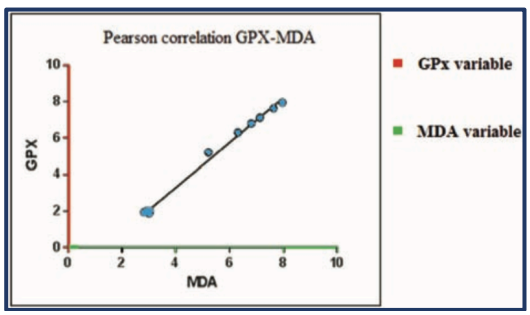

Fig. 12 Pearson correlation GPX-MDA.

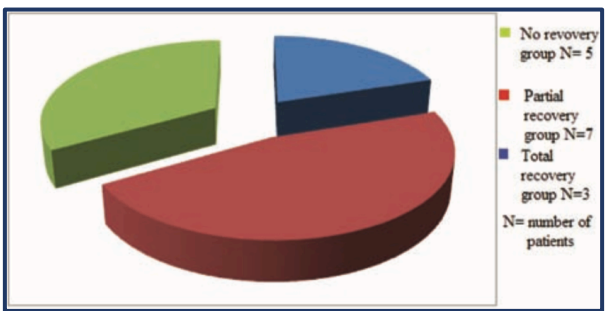

Fig. 13 Patients distrubution by hearing recovery level after corticoid treatment.

Recent studies have reported that the impaired microvascular perfusion occurring during an ischemic event in vascular disorders, including SSNHL, may be related not only to

traditional vascular risk factors such as hypercholesterolemia, hyperfibrinogenemia, hyperhomocysteinemia and microembolism, but also to oxidative stress, which may be synergic responsible for endothelial damage, especially in terminal microvascular systems (Capaccio et al., 2012).

We analyzed the influence of corticoid treatment on oxidative stress by measuring the activities of SOD and GPX before and after treatment. The beneficial role of corticosteroid therapy in stimulating the activation of SOD and GPX, antioxidant enzymes that neutralize NO^- was obvious.

In our study, corticoid treatment significantly decreased the MDA concentration. Early and efficient administration of corticoid treatment on patients diagnosed with SBI could block or at least ameliorate oxidative injury in the cochlear stria vascularis and thus achieve a satisfactory auditory rehabilitation.

The role of antioxidants in the prevention and management of hearing loss is still under debate. Targeting the oxidant response by antioxidants and modulating specific enzymes (e.g. NO synthases, NADPH oxidase) represent a potential therapeutic strategy; different clinical trials of antioxidant therapy (such as vitamin E, C, beta carotene, coenzyme Q10) have been proposed for main cardiovascular diseases but failed to show any convincing benefits (Mak and Newton, 2001). However, antioxidant therapy is often given not only for traditional cardiovascular diseases but also for otologic diseases such as tinnitus and slowly progressive sensorineural hearing loss (Hatano et al., 2008).

Medication with steroids and antioxidants may help in reducing the oxidative stress in the cochlea in SSNHL, implying a new direction in the treatment of this disease.

Adjuvant therapy based on antioxidant vitamins A, C, E. Vitamin A: reduces the concentration of the singlet oxygen and promotes the healing of the damaged ciliated cells. Vitamin E: reduces the concentration of the peroxyl radicals located in the cellular membrane. Vitamin C: helps the detoxification of free radicals during the aqueous phase. These 3 vitamins showed results in treating SSNHL (Hatano et al., 2008, Kaya et al., 2015)

Reviewing the literature regarding the use of antioxidant vitamins as a complementary treatment in SSNHL, one cannot say with certainty that these supplements lead to a higher precentage of recovering.

Accepted treatment remains cortisone despite the conclusions of a cochrane metaanalysis that suggests that the treatment with steroids in SHL is not proved to be efficient (Spear and Schwartz, 2011; Wei et al.,2013).

There is no unanimously accepted protocol regarding the dosage, the administration rithm and the substances used in treating SSNHL.

Liu et colleagues bring in the idea of following only one universal protocol which is both beneficial and forensic accepted (Liu et al., 2016).

A meta-analysis performed between January 1990 and July 2014 on 356 subjects - comprised 9 studies. Intratympanic cortisone administraton was compaired with systemic administration and, was shown that intratympanic cortisone administration results in a higher rate of complete recovery than systemic administration and it may be used when systemic corticotherapy is contraindicated. Intratympanic administration can lead to complications such as local infections, vertigo, lingual paresis etc. - but these complications are less frequent than those caused by systemic administration (Zhao, 2016).

The small size of our sample may be a limitation on the results statistical significance value. A larger sample was not possible because sudden deafness is a rare condition, and, on the other hand, some patients refused to participate in a study whose results will not affect their immediate hearing recovery. Further studies on a larger population could assist in confirming whether corticoids are implicated in mechanisms underlying the effect in hearing rehabilitation after sudden deafness.

Conclusions

In this study we describe the increase in antioxidant enzyme (SOD and GPX) activities and decrease in MDA concentration in ISSNHL patients after systemic corticoid treatment. Our results contribute towards the clarification of mechanisms which underlie the protective effect of glucocorticoids.

I.3.2 Optimization of hearing loss treatment with implantable devices

Background

Today, cochlear implantation is changing its indications and is attempting to improve the way a patient hears as compared to a conventional hearing aid.

More authors recommend personalized evaluation of every case establishing: the type and the localization of the lesion that led to hearing loss, the presence of some other comorbidities, the level of cognition, motivation, family´s support , individual expectation, etiology of deafness, morphology and size of the cochlea, residual hearing and so on.

Hearing does not have a fixed or absolute value, but rather, it is situated at different values in normal hearing parameters. The auditory threshold is represented by the weakest sound stimulus that can be perceived by the human ear. Hearing loss is defined by an auditory threshold shift with a decrease in hearing sensitivity.

Disabling hearing loss, based on 42 population studies, has a prevalence of 5.3% worldwide; statistically, 91% of those with hearing loss are adults and 9% are children (www.who.int/pbd/deafness/WHO_GE_HL.pdf; Stevens, 2013). According to the WHO, disabling hearing loss is defined as an increase of the auditory threshold in the hearing ear of more than 40 dB in adults (patients over 15 years of age) and of more than 30 dB in children (patients under 15 years of age).

Due to the nature of prelingual hearing loss, children have delayed language development, cognitive impairment, and low school performance (Fitzpatrick, 2015). In adults, hearing loss can lead to social isolation and difficulties in maintaining a job (Rydberg, 2010).

The treatment for hearing loss is dependent on multiple factors. First of all, the site of the lesion is important: at the level of external, middle and/or inner ear. In situations where the lesion is localized at the external ear (congenital aural atresia), external ear canal surgery can be attempted in accordance with the Jahrsdoerfer grading scale score. In order to recommend surgery a patient's score is calculated according to the Jahrsdoerfer system (Jahrsdoerfer et al., 1992). A score of 5 or less disqualifies the patient for aural atresia surgery.

Personal contribution

Scientific and professional achievements:

Based on experience gathered during more than ten years in the management of deaf persons using different auditory implantable prosthesis we have reviewed and analyze the outcomes of our cases. The main concern was to update the indications for implantable hearing aids according to the already obtained results. This study was published in some ISI and B.

Published papers:

Rădulescu L, Martu D
Do we need an ethics committee in order to make decisions regarding the cochlear implant? Revista romana de bioetica. 2007; 5(2):27-32. (ISI journal)

Mârţu D, **Rădulescu L**, Cozma S, Curcă AI.
Seven years of cochlear implant at the ENT Clinic of" Recuperare" Hospital Iaşi. 2008; 112(1):130-135.

Rădulescu L, Mârţu C, Mârţu D et al. Hipoacuzia - indicaţiile tratamentului cu proteze implantabile. ORL.ro, 2015; 8(27):22-25.

Cozma S, Manolache O, Olariu R, Mârțu C, **Rădulescu L**. Cochlear Implantation In A Child With Complex Bilateral Inner Ear And Cochleo-Vestibular Nerve Malformations. Romanian Journal of Oral Rehabilitation. 2015; 7(1):64-70.

Martu C, Cozma S, **Rădulescu L**, Martu D.
Objective tests for the evaluation of cochlear implant candidates. Romanian Journal of Oral Rehabilitation 2013; 5(2), 51-53.

3.2.1 Implantable prosthesis for the middle ear – Indications

Bone-active implants (BAI) require an external energy source which transforms input signals into mechanical energy that is transmitted directly to the ossicular chain or to the cochlea (Backous, 2006). BAI's can be totally or partially implantable and these different types use different forms of transductors to convert electrical stimulus into mechanical energy. This is represented by piezoelectric or electromagnetic vibrations with their specific electromechanical variations (Haynes, 2009).

The main indications for BAI are as follows:

- age over 18 years,
- moderate to severe SNHL,
- vocal audiogram with disyllabic words 50% or better

For candidates for VSB in who a transducer (FMT) has to be placed in connection with the ossicular chain (traditional coupled to the long process of the incus - but, new couplers have been developed that enable the FMT to be coupled to the short process of the incus)in both cases an anatomically normal middle ear is mandatory (Manrique, 2008). The main indications are for patients who cannot wear external earmolds that come with conventional hearing aids(due to atresia or stenosis of the external ear canal, chronic otitis externa or allergy to the material earmolds are made from), and for patients who have acoustic feedback. Every type of implantable device has its own specifications and specific auditory criteria. Benefits of BAI's are: correct sound amplification, no acoustic feedback, speech discrimination and no foreign body in the external ear canal (Manrique et al., 2008; Bouccara et al., 2005).

Bone-anchored auditory implants (Baha)

Bone-anchored implants transmit sound through bone to the cochlea through direct stimulation of the skull (Haynes et al., 2009).

Indications for this type of hearing device are: mixed hearing loss or conductive hearing loss in which ossicular reconstruction was not possible, radical open-cavity mastoidectomy, atresia or stenosis of the external ear canal or patients who cannot wear hearing aids for other medical reasons or who have not achieved sufficient benefits from other hearing systems and the bone conduction is better than 65 dB (Bouccara et al., 2005; Amonoo-Kuofi et al., 2015). Another distinct indication is single side deafness in which the auditory information is transmitted from the side of the deaf ear to the contralateral ear via skull vibration (Bouccara et al., 2005; Lek; ue et al., 2013; Manrique et al., 2008; Haynes et al., 2009).

3.2.2 Implantable prosthesis for the inner ear – expansion of indications

Cochlear implant is the first succesfull link between human brain and an electronic device. Hearing is the only sense, up until now, that can be restored. Cochlear implants have evolved exponentionally in the last few years. Since the 1970's when tens of patients benefited from implants, according to the Food and Drug Administration (FDA), as of December 2012, about 324,000 people have cochlear implants worldwide (in the United States about 58,000 adults and 38,000 children).

The cochlear implants are partial implantable devices. There is an external part – the sound processor, microphone, the antenna and the battery housing and an implantable part consisting in: receiver/stimulator, internal coil and the electrode array that enter the cochlea.
The microphone picks the environmental sounds. The sounds are prepared by the sound processor and the resulting information is transferred to the receiver / stimulator via the antenna. From the receiver /stimulator the stimulus travels through the electrode array to the neural elements by passing the cochlea.

In 1880, Volta observed that a current which passed through the mastoid at 50 V created a rumbling sound similar to that of boiling liquid.

A first step toward cochlear implantation was made by Djurno and Eyres in 1957 when a patient with a giant cholesteatoma perceived high frequency sounds after stimulating the patient's auditory nerve (Djourno and Eyres, 1957). Subsequently, in 1960s, House created a single-channel device and was later replaced by a multiple-channel device made by Simmons in US and by Clark in Australia paving the way to the development of the modern cochlear implants (House,1976; Clark et al., 2014). Back in 1960s, with the single-channel implants the patients were able to detect sounds and lip reading was easier and now, with modern implants people are able to understand speech and even to talk on the phone Bouccara et al., 2005; House, 1976) This spectacular evolution is a continuous one and thus indications for implants are constantly updated. Herein the classical norms and new indications for cochlear implants are presented based on the literature and personal experience.

Audiological parameters are of the utmost importance for cochlear implantation indications. In adults, according to the FDA, candidates have to have an average hearing loss of 70 dB or more, bilaterally (both ears), on frequencies of 500-1,000-2,000 and 4,000 Hz, the tonal auditory threshold in free field with hearing aids have to be greater than 55 dB on the same frequencies and disyllabic discrimination value of less than 40% on the speech audiogram in free field with hearing aids at an intensity of 65 dB (the hearing aids have to be worn a minimum of 6 months before cochlear implantation approval).

The audiological criteria in children are different than those for adults due to the fact that behavioral tests are difficult to be done and interpret and due to a lack of tests that can

examine the entire range of frequencies. In this case, the FDA recommendations for children that are candidates for cochlear implantation are the following: SNHL when the average auditory threshold is calculated on frequencies 500, 1000, 2000 and 4000 Hz and is more than or equal to 90dB (Sampaio et al., 2011).

Furthermore, from the possible candidates for cochlear implantation, those with acute or chronic inflammation of the middle or external ear are excluded. Once the inflammatory process heals, the patient can be considered for cochlear implantation. Other contraindications for cochlear implantation are: cochlear aplasia, cochlear nerve agenesis, cochlear ossification (Manrique et al. 2019).

Candidates for cochlear implantation undergo rigorous examination to determine the cause of hearing loss and to establish the extent to which it may hinder cochlear implantation, for example:

- In temporal bone fractures, the facial nerve could be stimulated during implant activation and the stimulation parameters would need to be modified;
- Fibrosis or ossification of the cochlear duct after meningitis changes the procedure of cochlear implantation;
- Patients with neurofibromatosis type II and those with the cochlear nerve aplasia may need a brainstem implant.

Age is not an impediment for cochlear implantation. Currently, patients younger than 12 months have been implanted and as old as 84 to 86 years; however, the usual age limit is 95 years (Lin, 2012).

Indications for cochlear implants in children have changed along the years as well (Nicholas and Geers, 2007; Hang et al., 2012). Until 1990's, only adults were accepted as candidates for cochlear implantation and now, children have a higher priority and are the favored candidates.

The auditory system and the child's brain are genetically pre-configured but their development is modulated by the external environment being either restrictive or permissive. Speech development starts from birth and is almost completely developed by the time the child reaches the age of 6 years. Due to this fact, children with congenital deafness need to be implanted when cerebral plasticity is at its greatest. After the ages of 5 to 8 years, the chances of speech development decrease significantly (Kral and O`Donoghue, 2010; Colleti et al., 2011). Until 2000, the smallest recommended age for implantation was 2 years and after 2000, the FDA approved cochlear implantation starting with the age of 12 months. Implantation in younger infants is now accepted although audiological and anesthesiologic implications are still in debate. In hearing loss due to meningitis cochlear implantation is considered as an emergency (Philippon et al., 2010).

The success of rehabilitation after cochlear implantation is strong corelated with a good familial and community support and with maternal education degree.

The remarkable progresses made in implant technology led to the implementation of certain selection criteria of the candidates and thus, patients that would not have been able to benefit from a cochlear implant years ago can now be accepted as valid candidates for cochlear implantation, according to the literature.

The current indications refer to bilateral cochlear implantation, especially in cases of SNHL post-meningitis or in cases of concomitant blindness. Bilateral cochlear implantation improves sound localization and speech discrimination in noise (Härkönen et al., 2015). It is a delicate decision whether to bilaterally implant a child simultaneously or sequentially at a certain interval between the two. The preferred approach has still not been established (Peters et al., 2007; Gordon and Papsin, 2009).

Cochlear implantation in unilateral deafness has been gaining ground recently. Studies in which cochlear implants are compared to the traditional hearing aids used for unilateral

hearing loss (CROSS or IAO) show to be efficient (Blasco and Readleaf, 2014). Moreover, in the majority of studies on unilateral hearing loss with tinnitus, electrical stimulation plays an important role as a tinnitus reliever. In most cases, tinnitus is suppressed (46-95% of cases) with only 5.6% of cases reporting an aggravation of tinnitus after electrical stimulation (Todt et al., 2015; Olze, 2015).

Auditory neuropathy (AN) is group of disorders that have an incidence of 2.4-15% in children diagnosed with SNHL (Hang et al., 2012; Roush et al., 2011). A study comprising 260 children diagnosed with AN show that 5% of these children developed normal speech without the use of hearing aids or other auditory prostheses (Berlin et al., 2010). The results of children with AN that were implanted were variable (Teagle et al., 2010).

Conclusion

Implantable hearing aids came about as a necessity in cases where medical or surgical treatments could not be done or have failed or in cases who do not benefit from conventional hearing aids.

Today, cochlear implantation is changing its indications and is attempting to improve the way a patient hears as compared to a conventional hearing aid.

I.3.3 Optimization of cochlear implant surgical technique

3.3.1 Optimization of cochlear implant selection - measurement of the cochlear duct

Background

In children with profound hearing loss, electrical stimulation of as many as possible neural elements along the cochlear duct is important for optimal speech and language rehabilitation. On the contrary, in cases with residual hearing, conservation of the functional part of the cochlea impose partial insertion of the electrode array (Snels et al, 2019).

There is a variability in the size of the cochlea (Zahara et al., 2018), to correctly choose the length of the electrode array, the solution is to measure the length cochlear duct in each CI candidate.

On the market there are available electrodes of different lengths to aloud to choose the correct electrode size for each patient. Proper selection of electrode is important in both: to cover all the length of the cochlear duct and also to avoid insertional trauma (Gstoettner et al., 1997; Landsberger et al., 2014): Contour Advance, Slim Modiolar, Slim Straight, Hybrid L24, and Straight Full Banded from Cochlear Limited (Sydney, Australia), FLEXSOFT, FLEX28, FLEX24, FLEX20, FLEX16, FORM24, FORM19, STANDARD, MEDIUM, and COMPRESSED from MED-EL (Innsbruck, Austria), HiFocus 1J, HiFocus Helix, and HiFocus Mid-Scala from Advanced Bionics (Sonovo, Switzerland), Neurelec EVO from NEURELEC Medical (William Demant, Danemarc) (Frisch et al., 2015; Zeng et al., 2015).

Knowing both, the electrode location as well as the morphology and size of the cochlea, is required to obtain more reliable estimations of the voltage distribution and the neural activity of each CI user. (Malherbe et al., 2015).

Beside the cochlear duct length variability, there are cases with malformed cochlea (Fig 14 and 15) or with cochlear obliteration that impose, moreover, a correct choice of the type and length of the electrode. (Fig.16)

Proper implantation requires that all electrode contacts cover the entire length of the cochlea without causing any lesions inside the cochlea (Downing, 2018).

So, the electrode array must not be too big or too small, but its length should be as close as possible to the length of the cochlear duct.

One of the main methods of achieving customized surgical implantation is choosing the length of the electrode array according to the size and shape of the cochlea of each person to be implanted.

There are three widely accepted reasons for selection of an appropriate length of the electrode array:

- More accurate correspondence between the frequency spectra of processed sounds and the location of electrical stimulation – the better the speech understanding.
- Improvement of efficiency of the electrode stimulation by positioning it closer to the cell bodies of spiral ganglion neurons / to surviving peripheral nerve fibers within the osseous spiral lamina.
- Less trauma to the delicate structures of the inner ear during insertion of the electrode array – the more neural elements will survive to the electrode insertion.

The length of the cochlear duct (CD) is considered from the middle of the round window to the helicotrema and is fully formed at birth.

In 1938 Hardy, performed first histologic measurements of the CD length in 68 bodies. He used graphic reconstructions of serial sections for his measurements (Hardy, 1938).

Since the advent of cochlear implantation, multiple studies have assessed the relevance of using the CDL for selection of the appropriate electrode length for implantation. This is particularly important as the length of the cochlea may vary greatly from one person to another (between 25 – 45 mm) (Dimopoulos and Muren, 1990; Kawano et al., 1996; Lee et al., 2010; Erixson et al., 2009; Avci et al., 2014) (Table VIII).

Table VIII – Measurements of CDL made by different authors (Lee, 2010)

Comparing CDL measurements made by multiple studies

Authors	Year	Location of CDL	Modality	Method	# of Samples	Mean (SD)	Range of Values
Retzius	1884	OC	Histology	Direct	5	33.5 (0.8)	32 – 34
Hardy	1938	OC	Histology	Indirect	68	31.52 (2.3)	25.26 – 35.45
Bredberg	1968	OC	Histology	Direct	35	34.0 (1.3)	30.3 – 37.6
Walby	1985	OC	Histology	Indirect	20	32.6 (2.1)	30.1 – 36.4
Ulehlova et al.	1987	OC	Histology	Direct	50	34.2 (2.9)	28.0 – 40.1
Pollak et al.	1987	OC	Histology	Indirect	9	28.4 (3.4)	24.0 – 33.5
Wright et al.	1987	OC	Histology	Direct	14	32.9 (2.6)	28.8 – 36.6
Takagi & Sando	1989	OC	Histology	3D reconstruction	1	36.4 (n/a)	-
Sato et al.	1991	OC	Histology	3D reconstruction	18	34.73 (2.9)	29.7 – 38.9
Kawano et al.	1996	OC	Histology	3D reconstruction	8	35.58 (1.4)	34.2 – 37.9
		LW	Histology	3D reconstruction	8	40.81 (2.0)	37.93 – 43.81
Ketten et al.	1998	OC[a]	In vivo CT	Spiral coefficients	20	33.01 (2.3)	29.07 – 37.45
Skinner et al.	2002	OC[a]	In vivo CT	Spiral coefficients	26	34.62 (1.2)	32.94 – 36.57
Sridhar et al.	2006	OC	Histology	Direct	7	33.31 (2.4)	30.5 – 36.87
Stakhovskaya et al.	2007	OC	Histology	Direct	9	33.13 (2.1)	30.5 – 36.87
Erixon et al.	2009	LW	Plastic casts	Indirect	58	42.0 (2.0)	38.6 – 45.6
Lee et al.	2010	OC	Histology	Indirect	27	30.8 (2.6)	25.5 – 35.1

[a]Measured LW and interpolated into the OC location

Aim of the study

Starting from the assumption that the study group with the 35delG mutation is homogenous having small variation of the CDL between the group members, **the aim of the study** was to compare:

- the length of the cochlear duct of CI candidates with 35 delG mutation on GJB2 – study group,

with

- the length of the cochlear duct of CI candidates without any mutation on GJB2 – control group.

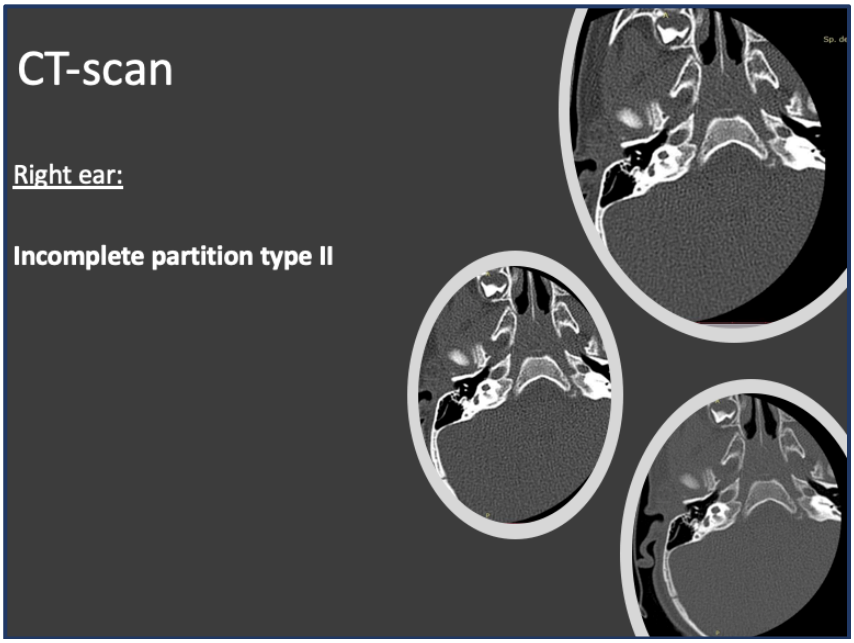

Fig.14 Malformation of the right cochlea (incomplete partition type II) in a bilateral deaf child candidate to cochlear implantation

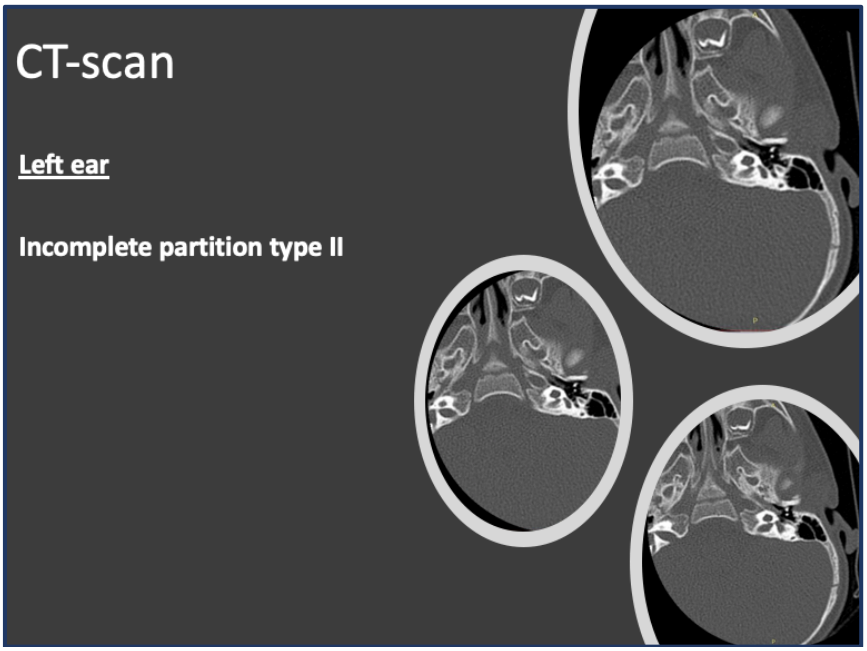

Fig. 15 Malformation of the left cochlea (incomplete partition type II) in a bilateral deaf child candidate to cochlear implantation

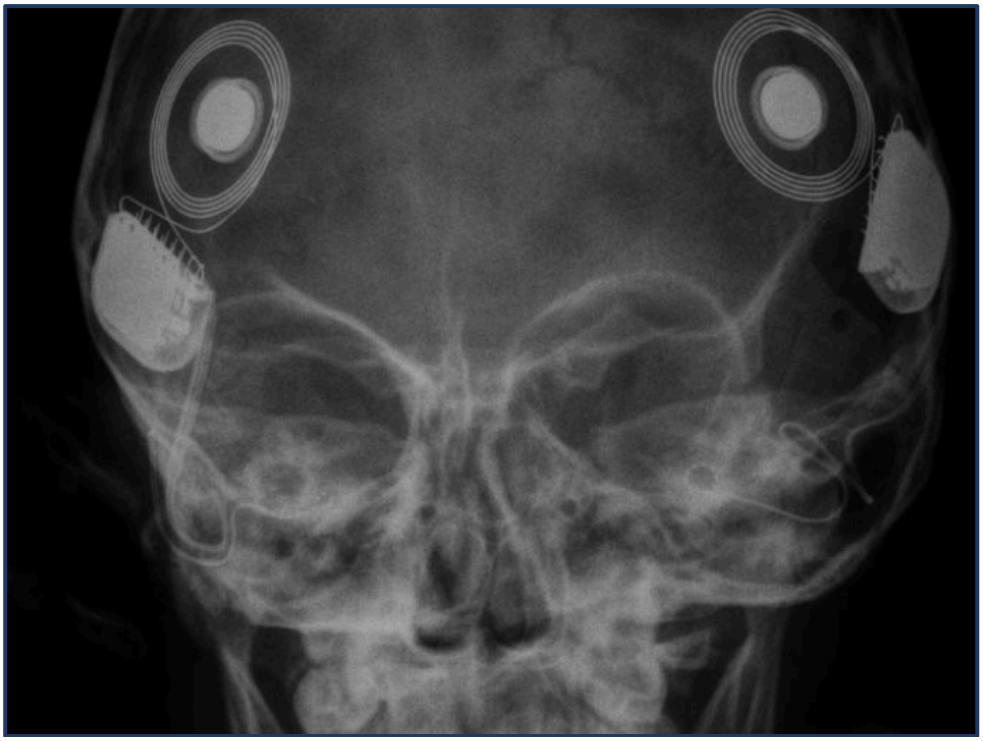

Fig. 16 Bilateral cochlear implantation in a child with bilateral malformation of the cochlea

Material and methods

- Control group: CI candidates without GJB2 mutations – 22 ears;
- Study group: CI candidates with 35delG mutations – 20 ears;

We had measured 10 parameters: Modiolus / Endolymphatic fossa / Vestibular aqueduct / Posterior semicircular canal (PSCC) width / PSCC length / Cochlea width / Cochlea height /

Cochlear nerve / Internal auditory canal / Insertion angle;

- The measurements of coronal cochlear height and axial LSCC bony width have excellent reproducibility and identify bony labyrinth abnormalities missed by visual inspection alone.
- Two temporal bone computed tomography measurements made by different people increase the accuracy of malformations recognition Measurement of the diameter of the first tour of the cochlea (Purcell, 2006).
    - CDL = 4.16A-3.98 - where A is the diameter of the first tour of the cochlea.
    - A was measured from the round window to the farthest point on the opposite wall of the cochlea on a reformatted CT scan slice (Grover, 2018).

Results

Results are depicted in the Table VIII-XII and in Figure 17 and 18.

We applied Kruskal-Wallis Test:
The p-value for the cochlear height was .33667. The result being not significant at $p < .05$
The p-value for the cochlear length was .67317. The result being not significant at $p < .05$.
The p-value for the cochlear height was .77599. The result being not significant at $p < .05$

Table IX - Parameters measured in the control group - without 35delG mutation

	modiolus	fosa endolimf	LVA	CSP Latime	CSP Lungime	Coch length	Coch height	n. coch	IAC
1	1,2	1,3	0,6	0,6	7,2	8	4	1,7	4,5
	1,1	1,1	0,6	0,6	7,1	8,1	4,1	1,5	4,6
2	1,2	1,1	0,4	0,6	6	8,5	4,4	1,5	6,3
	1,1	1,1	0,4	0,6	6	8,4	4,3	1,5	6,2
3	0,9	0,9	0,6	0,6	6	8,2	4	1,1	5,7
	0,9	0,8	0,5	0,5	5,5	8,1	4	1,1	5,7
4	0,9	1,3	0,7	0,6	7	8	4,3	2	4,4
	0,9	1,4	0,7	0,6	7,2	8	4,3	1,6	5
5	1,1	1	0,6	0,6	5,8	7,3	4,2	1,4	6,1
	1,1	1	0,6	0,6	5,7	7	4,2	1,6	7,1
6	1	1	0,5	0,6	6,8	7,3	3,6	1,7	5,3
	1	1	0,5	0,6	6,7	7,7	3,8	1,6	5,1
7	1,1	1,2	0,5	0,5	6,1	8,2	4,3	1,3	4,6
	1,1	1,2	0,5	0,5	6,2	8	4,3	1,6	5
8	1	1,1	0,4	0,7	5,8	7,3	3,6	1,4	4,3
	1	1,2	0,5	0,6	5,5	7,1	3,6	1,3	4,6
9	1,1	1,1	0,5	0,5	5,8	8,2	4,5	1,5	5
	1,1	0,9	0,4	0,6	5,9	8,2	4,5	1,5	4,4
10	0,9	1,1	0,5	0,6	6,3	7,7	3,9	1,3	5,4
	1	1,3	0,6	0,6	6,1	7,8	4,2	1,3	5,2
11	1,1	1,1	0,5	0,7	6,8	8,3	4,3	1,8	3,8
	1,2	1,2	0,7	0,6	7	8,1	4,3	1,7	4,2

Table X - Parameters measured in the study group - with 35delG mutation

	modiolus	fossa endolimf	LVA	CSP Latime	CSP Lungime	Coch length	Coch height	n. coch	IAC
1	0,9	1,2	0,6	0,6	8,2	7,5	4,5	2,1	5,2
	0,9	1,5	0,6	0,6	8,1	7,3	4,4	2,1	5,4
2	0,9	1,2	0,6	0,6	7,5	7	3,8	1,8	4,1
	1	1,2	0,5	0,7	7,8	7	3,8	1,8	5,1
3	1	1,8	0,6	0,7	7,2	7,8	3,8	1,7	4,2
	1	1,8	0,7	0,7	7,2	7,6	3,7	2	4,7
4	0,9	0,8	0,3	0,6	7	7,5	4	2	5
	1	0,8	0,4	0,6	6,5	7,2	4	1,7	3,4
5	1,2	0,9	0,5	0,6	6,3	8	4,1	2,1	5,2
	1,1	0,9	0,5	0,7	6,5	8	4,2	2,1	5,2
6	1,2	1,3	0,6	0,6	6,5	8	4,5	2,1	4,6
	1,4	1,3	0,6	0,7	6,5	8	4,6	2,1	4,5
7	0,9	1	0,6	0,7	4,5	5,4	3,3	1,4	3,8
	1	1,1	0,6	0,6	4,7	5,6	3,5	1,4	4,2
8	1,2	1	0,5	0,6	6,3	7,8	4,2	1,9	4,5
	1,2	0,9	0,5	0,6	6,3	7,5	4,4	1,8	4,5
9	1,2	0,6	0,3	0,7	7,3	9,3	4,3	1,7	4,3
	1,1	0,8	0,5	0,7	7,3	9	4,3	1,9	4,4
10	1	0,9	0,4	0,7	7,4	8	4,3	1,4	4,3
	1	0,9	0,5	0,6	7	7,7	4,2	1,6	4,4

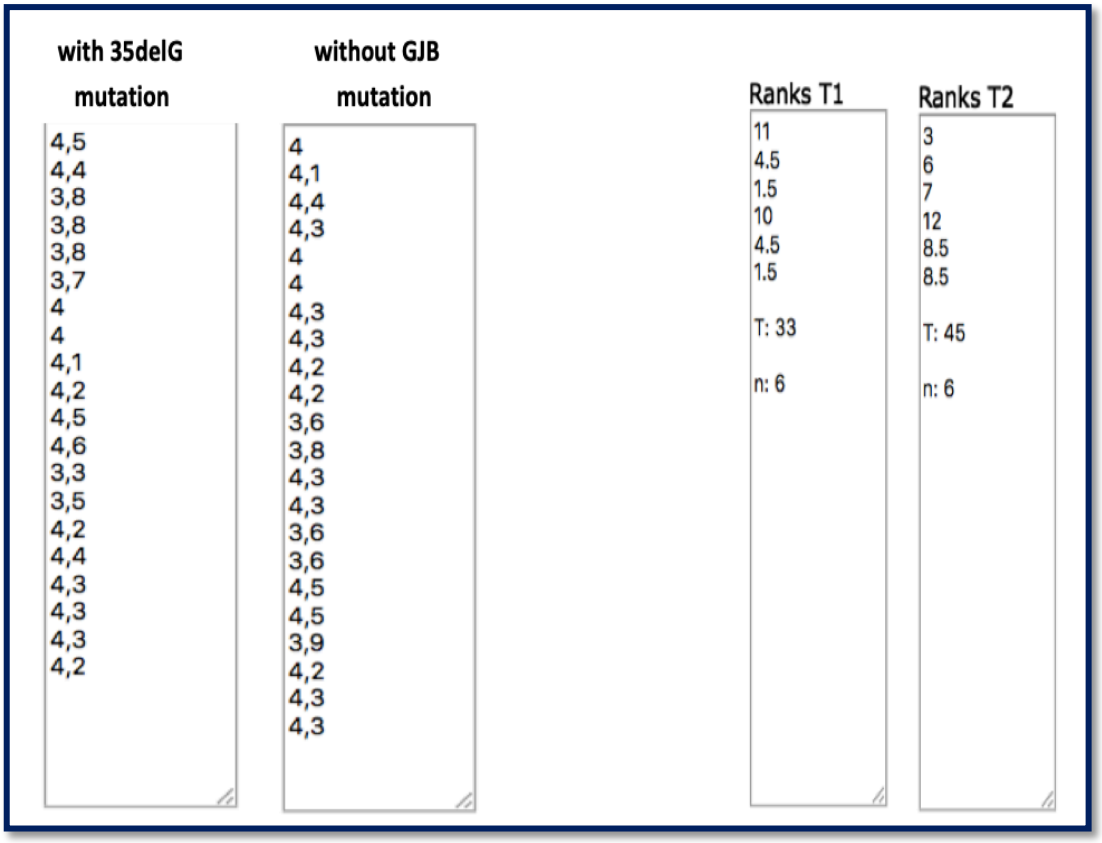

Fig. 17 Size of the cochlear duct in subjects with vs without GJB2 mutation

Table XI – Comparing measurements of CDL in cases with and without GJB2 mutation

Without GJB2 mutation	With 35delG mutation

• cochlear width: 8.5-7
  • Median: 8
  • Mean: 7.88
• cochlear height: 3.6-4.5
  • Median: 4.1
  • Mean: 4.12
	• cochlear width: 5.4-9.3
	  • Median: 7.6
	  • Mean: 8.1
	• cochlear height: 3.3-4.6
	  • Median: 4.1
	  • Mean: 4.01

Mean = 4.12 mm Median = 4.1 mm Range: 3.6 to 4.5 mm	Mean = 4.01 mm Median = 4.1 mm Range: 3.3 to 4.6 mm

DIsscusions

The size of the human cochlea – and the most important – of the cochlear duct has been extensively studied especially after the multichannel CI development (Koch RW et al., 2017). It was found that there are considerable variations of CD size between individuals. The length of the cochlear duct can vary between 25 – 45 mm.

In addition, in 10 % of cases, cochlear malformations occur. (which require individualized surgery more than in any other situation, because the length of the electrodes array has to be in accordance to the size of the cochlea (Fig.14 and Fig.15).

Cochlear duct length has been an important measure for the development and advancement of cochlear implants. Emerging literature has shown CD can be used in preoperative settings to select the proper sized electrode and develop customized frequency maps. In order to improve post-operative outcomes, and develop new electrode technologies, methods of measuring CDL must be validated to allow usage in the clinic

Another parameter important for electrode selection is the insertion angle (Table XII) and (Fig. 18).

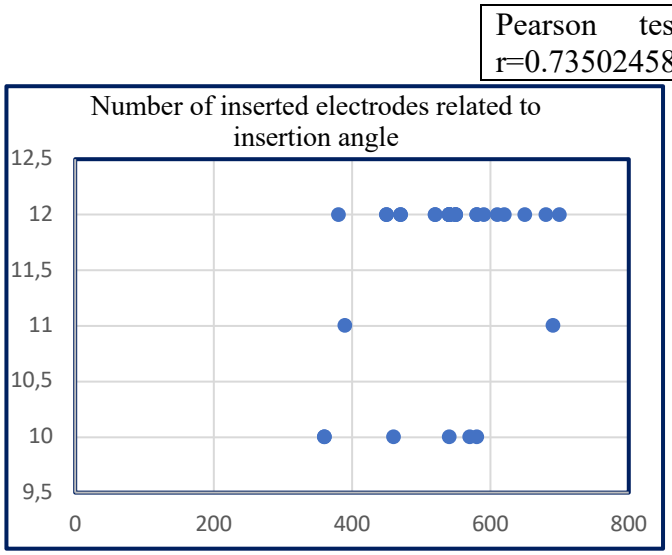

Fig. 18 Correlation between the angle of insertion and number of inserted electrodes in the cochlea

Consonant-nucleus-consonant scores trend appears to plateau once the insertion depth passes 450 degrees, suggesting that this insertion depth is deep enough to expect maximal outcomes (Rivas, 2017).

Thus, our strategy would be to choose an electrode array that is long enough to at least reach a 450 degree depth at full insertion and short enough to permit having all basal electrodes inserted into the cochlea when the tip insertion depth reaches 560 degrees.

The presence of 35delG mutation does not affect the length of the cochlear duct so, the measurement of the cochlea has to be done to assure the selection of the appropriate electrode array in each and every case.

The size of CD has considerable variation and CI electrode array has to be choose in accordance.

Simple visual evaluation of the CT scan may be not enough.

Conclusions

Manual measurements are an alternative for those that do not have the possibility of automatic measurements.

Table XII Angle of insertion and number of inserted electrodes

Patient	Insertion angle	Number of electrodes
pi	550	10
ceg	460	10
ma	360	10
ma	540	10
vd	360	10
ce	580	10
bl	570	10
nc	690	11
ti	390	11
sm	540	12
sm	520	12
cv	540	12
cv	550	12
ii	450	12
ii	470	12
ch	620	12
pe	540	12
pe	540	12
bv	580	12
bv	580	12
cm	540	12
cm	520	12
dc	550	12
dc	550	12
pd	380	12
pd	450	12
vn	590	12
vn	470	12
op	680	12
sa	700	12
sa	540	12
tm	610	12
ce	650	12
oa	540	12

3.3.2 Optimization of cochlear implant surgical technique

Background

In March 2000, the first cochlear implantation was done by our team (Professor Dan Martu and I) in Iași. At that time, there was already a global experience in this field; there were two types of incisions and both had already been rejected.

In the House Ear Institute, most complications that occurred were due to the "C" flap with anterior pedicle (Fig. 19) (Thielemeier, 1985). There were other authors that reported complications at the surgical site secondary to this type of incision (Harris and Cueva, 1987). The most complications reported were connected to the flap (0.6-2%).

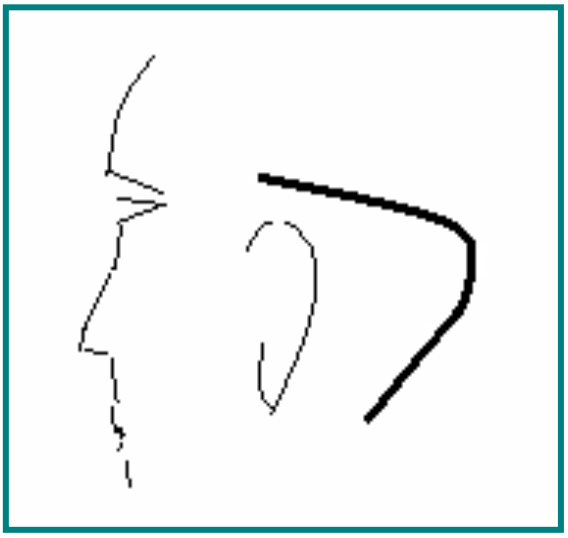

Fig.19 "C" flap with anterior pedicle

Fig. 20 "U" flap – pedicle based on occipital artery

Complications ranged from major, in which the implant needed to be explanted, to minor complications such as superficial infections, air trapped under the flap, seroma and keloids.

The probable causes of these complications were: an incision placed too anteriorly, a narrow flap base, the inferior portion of the incision situated under the auricle with the interception of the posterior auricular artery (Cohen et al., 1988; Schweitzer and Burtka, 1991). Thus, in Melbourne, a new incision came about, this time in an inversed "U" shape with the pedicle placed inferiorly at the occipital artery level (Clark et al, 1979); this incision was used at Hannover (Fig. 20). Some authors reported flap complications (Webb et al., 1991) while other authors reported no complications with this type of incision (Schweitzer and Burtka, 1991). Even this incision type is no longer used today. The endaural incision that extends posterior-superiorly (Fig. 21a and b) was the most used incision in March 2000 when our team was undergoing our first cochlear implant surgery. This type of incision proved to have fewer complications (flap necrosis, suture failure, wound infections, hematoma and seroma) (Telian et al., 1999; Aschendorff et al., 2005). The major problem with this incision was aesthetic aspect (Fig. 21b) and the long time it took to make a flap.

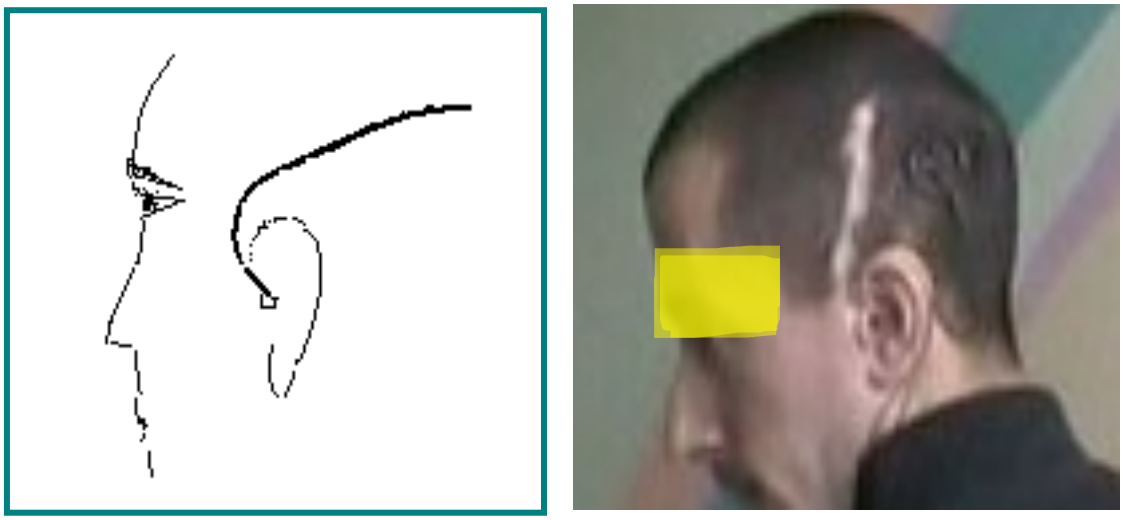

Fig. 21a and b Endaural incision that extends posterior-superiorly

The following incision type was a retroauricular incision extended superiorly and posteriorly (Fig. 22 a and b). Even though it was more aesthetically compared to the endaural approach, the extension of the incision left the same scars as the other approaches because in that area the incision remained the same. Complications occurred, as anteriorly mention in the previous incision types, however, their number was reduced (Telian et al., 1999).

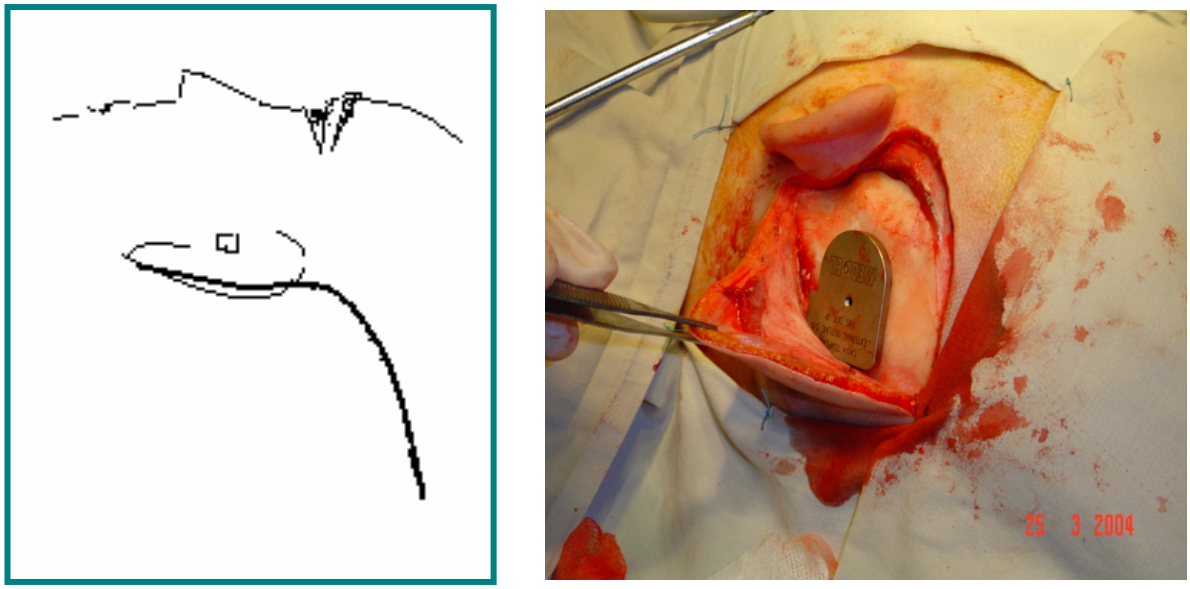

Fig. 22a and b Retroauricular incision extended superiorly and posteriorly

In order to reduce significantly the complication rate, diverse authors (O'Donoghue Nikolopoulos, 2002; Jiang et al., 2004; James and Papsin, 2004) proposed a short retroauricular incision, slightly concave anteriorly and a tight subperiosteal pocket created for the receiver-stimulator posteriorly (Fig. 23). Using this technique, the number of complications decreased notably (O'Donoghue, 2002; Jiang et al., 2004; Aschendorff et al., 2005) because a retroauricular incision does not hinder major local vascularization (Pau et al., 2004) and aesthetically, the post-operative aspect is greatly improved (Fig. 24 a and b).

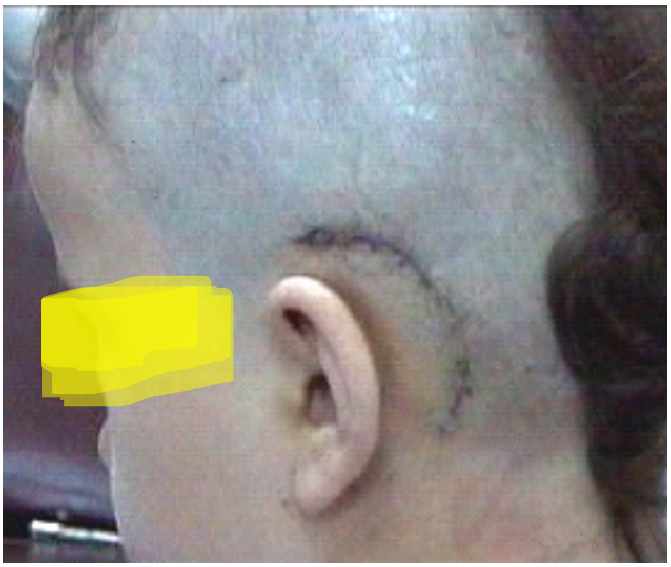

Fig. 23 Short retroauricular incision, slightly concave anteriorly

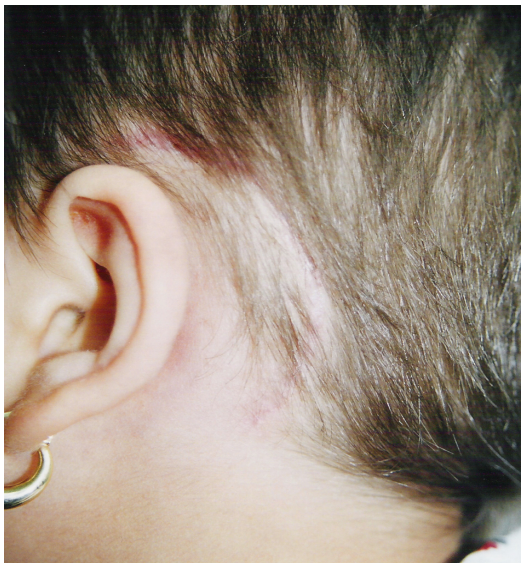

Fig. 24a Post-operative aspect

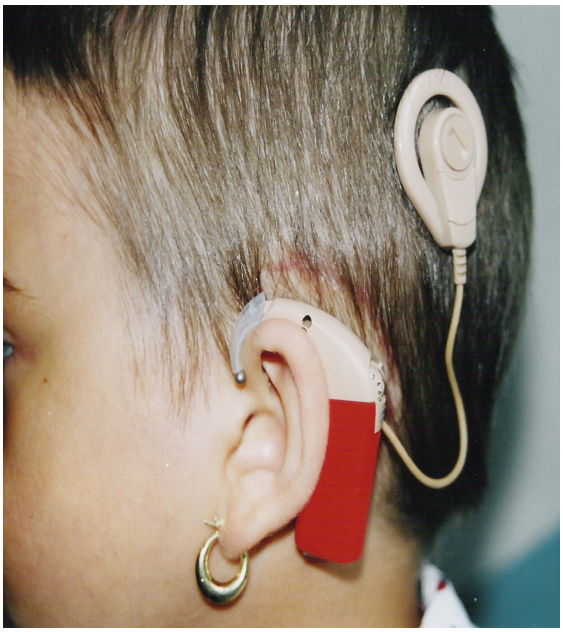

Fig. 24b Aesthetically, the post-operative aspect is greatly improved

Conclusion

The only problem that remains is the risk of receiver-stimulator displacement. The results of various authors (Aschendorff et al., 2005) that have carried out studies on large groups over a longer period of time are disputed. Some authors (Aschendorff et al., 2005) have not reported the displacement of the receiver-stimulator while others (James and Papsin, 2004) consider this an imminent risk. To solve this problem, it was decided to sew the receiver-stimulator together to the tight periosteal pocket.

3.3.3 New device for cochlear implant surgery

Background

To optimize access to retroauricular area where receiver stimulator has to be fixed through a minim incision, we patented a device called **"Device for cochlear implant surgery"**.

PATENT / BREVET

Retractor for performing cochlear implant surgery, has tubular medical steel rod that is welded under specific angle so as to support detachable lighting system which is connected to cavity through orifice.

Patent Number: RO128260A2; RO128260B1
Patent Assignee: **RĂDULESCU LUMINIȚA**; MÂRŢU C
Inventor(s): **RĂDULESCU LUMINIȚA**; MÂRŢU C.

The device is comprised of a retractor that maintained surgical access to the location of the receiver/stimulator.

Our device was meant to replace the Langenback retractor used to enlarge the acces to the surgical field. The device is made up of a metallic spatula and a knob to ease the retractor handling. A disadvantage of this retractor is that it has to be held with one hand at all times which makes the surgeon's job more difficult. Another disadvantage is that it takes effort to maintain the retractor within the surgical site which hinders surgical precision.
Furthermore, another disadvantage is that the Langenback retractor does not have an integrated light source.

The problems solved by this invention is that, being dedicated for cochlear implant surgery allows an efficient and precise surgery and gives to the surgeon the possibility to work with both hands in a well-lit surgical site.

This device overcome the mentioned drawbacks in that it ensures a protected and uniformly lit surgical field due to a detachable light source that is located at a 60° angle and connected to an optic cable. This detachable light source is attached to a cylindrical shaft that also permits the manipulation of the device. Also, the entire device is made from medical stainless steel in the shape of a wedge with the tip angled at 30°. The tip is inserted under the skin through retroauricular incision.

This proposed tool has the following advantages:

-it is easy to be manufactured and is reliable
-its usage does not require supplementary instruction
- shortens the duration of surgery

Thus, in the following there is an example of how the invention is used also in relation with figure 25 that represent:

Figure 25 - The device according to the invention is made from one piece of medical stainless steel (1) in the form of a wedge with an angled tip $\alpha = 30$ ° in relation in the upper part of the device with a tubular rod (2) of medical stainless steel welded to the piece one(1) at an angle of $\beta = 60$ °. The two lateral walls and the upper part of the piece one (1) define an open cavity (a) that assures a protected surgical field.

The tubular rod (2) allows both the manipulation of the device and the accomodation of a lighting system (5) using a clamp (3) with a jagged screw (4) to illuminate the cavity (a), through an orifice (b), thus ensuring optimum visibility in the operating field.

The lighting system (5) is connected through an optical fiber to a specialized light source.

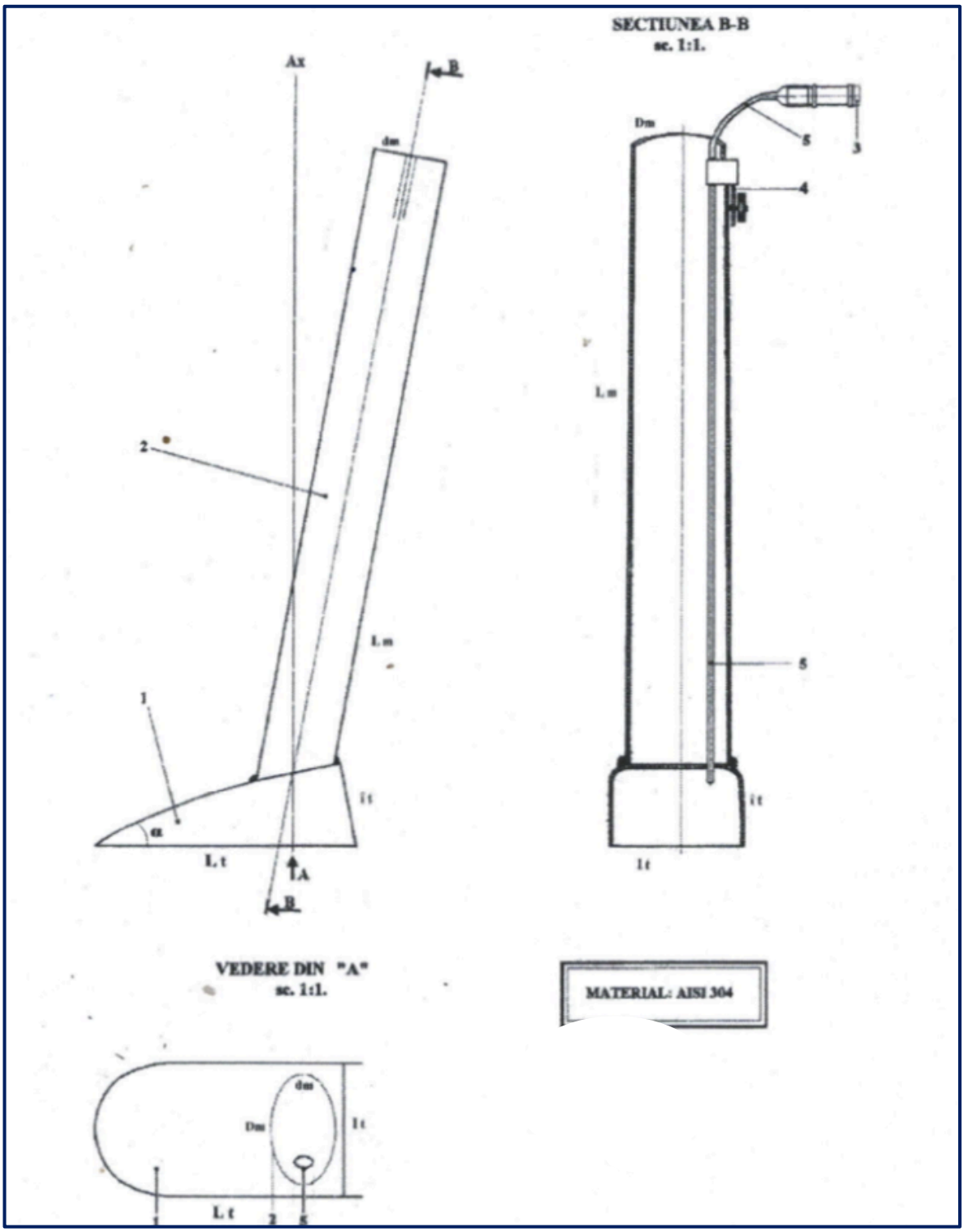

Fig. 25 Retractor for performing cochlear implant surgery with two sections: vertical one through the handle (section B-B) and a horizontal one through the retractor A-A

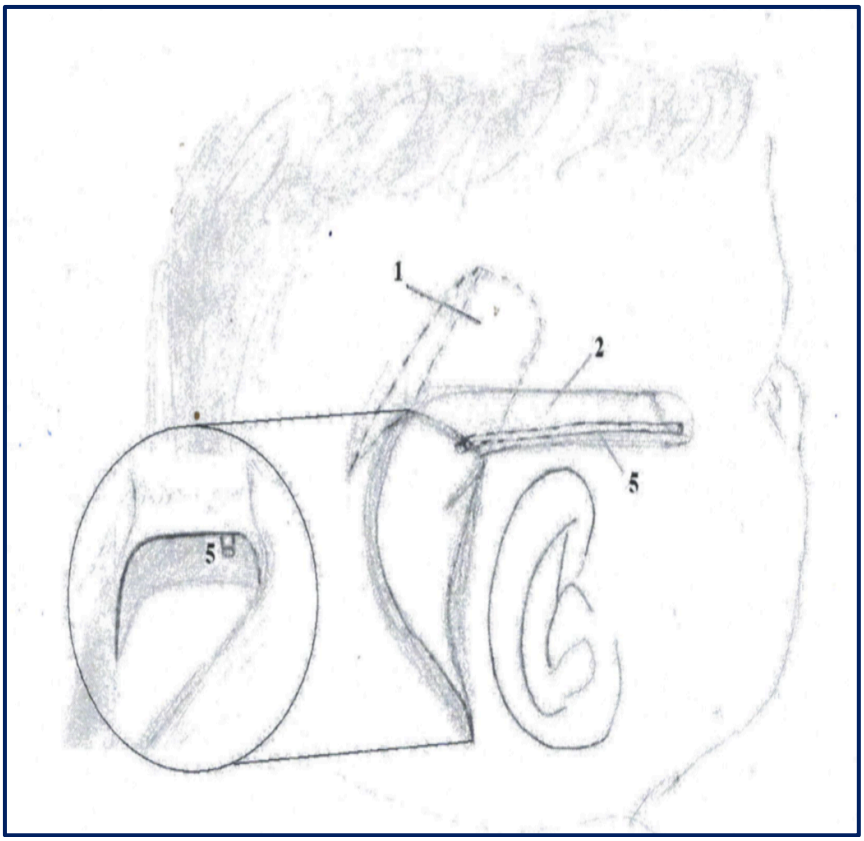

Fig. 26a The retractor under the scalp delimitating the surgical field for drilling the bed of the receiver /stimulator

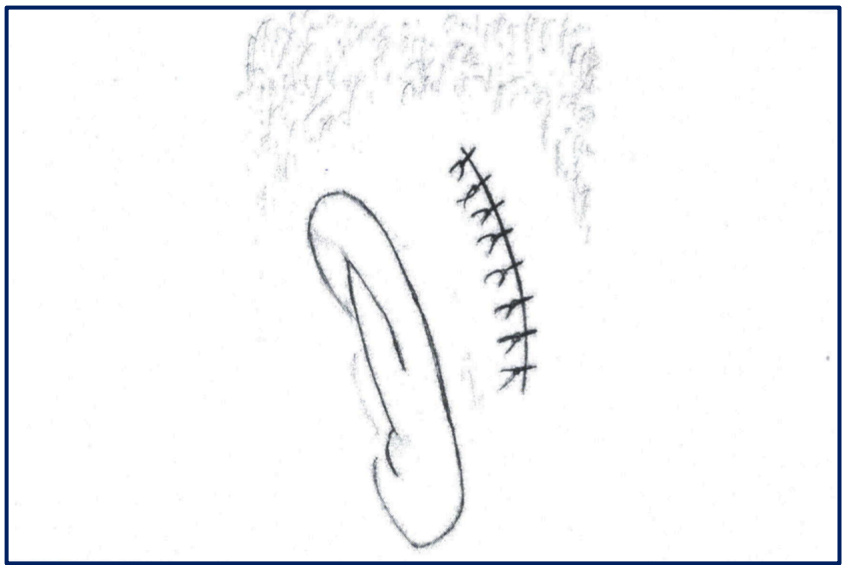

Fig. 27a Small incision used with the illuminated retractor for drilling the bed of receiver/stimulator

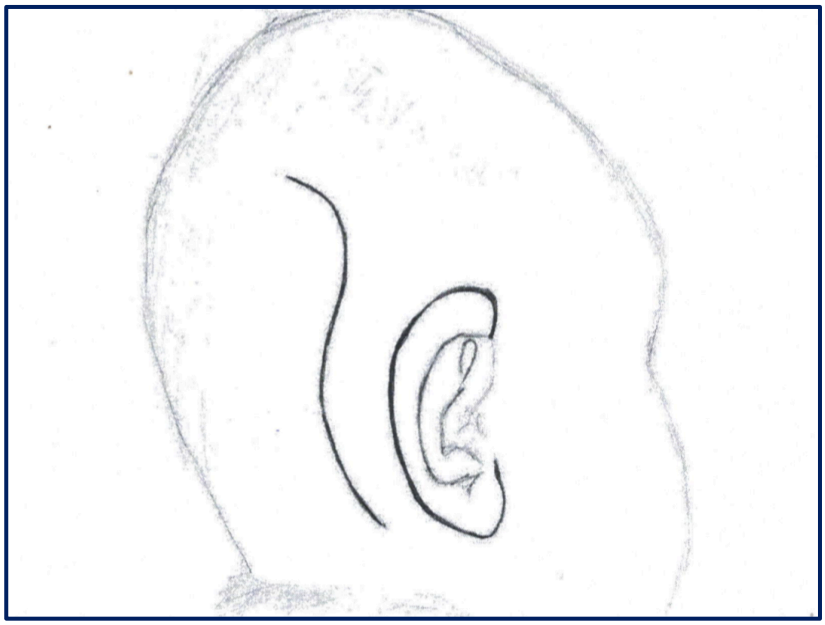

Fig. 28 The large incision anteriorly used in Cochlear Implant surgery.

ROMÂNIA

DUPLICAT

ELIBERAT COTITULARULUI BREVETULUI DE INVENȚIE NR. 128260

OFICIUL DE STAT PENTRU INVENȚII ȘI MĂRCI

Brevet de invenție

Nr. 128260

Acordat în temeiul Legii nr.64/1991 privind brevetele de invenție, republicată în Monitorul Oficial al României, Partea I, nr.613, din 19 august 2014.

Titular: RĂDULESCU LUMINIȚA, IAȘI, IS, RO; MĂRȚU CRISTIAN, IAȘI, IS, RO

Titlul invenției: DISPOZITIV PENTRU INTERVENȚIA DE IMPLANT COHLEAR

Inventatori: RĂDULESCU LUMINIȚA, IAȘI, IS, RO; MĂRȚU CRISTIAN, IAȘI, IS, RO

Descrierea invenției, revendicările și desenele la care se face referință în acestea, fac parte integrantă din prezentul brevet de invenție.
Durata brevetului de invenție este de 20 ani, cu începere de la data de 01/09/2010, cu condiția plății taxelor anuale de menținere în vigoare a brevetului.

Confirm cele de mai sus prin semnarea și aplicarea sigiliului
Director General

OFICIUL DE STAT PENTRU INVENȚII ȘI MĂRCI • ROMÂNIA •

București. Data eliberării 30/03/2015

Fig. 29 Invention certificate for illuminated retractor for cochlear implant surgery

For perfect sterilization of the device both the piece one (1) and the tubular rod (2) are chrome plated.

For the purpose of surgery with the device according to the invention it will be placed under the scalp where the receiver-stimulator pocket has been made after which the light source

is connected. This creates a surgical field under the scalp where the bed of the receiver/stimulator can be drilled. Surgery can be performed using both hands with a clear view of the surgical field due to the illumination system (5).

Description of the surgical technique

A small retroauricular incision (3-4 cm) is made exposing the external face of the mastoid process. Before this modern incision, the incisions were large enough to permit a good view of the operating field but the scar was large (about 10-15 cm on the lateral side of the scalp) and the flap morbidity was quite important (Figure 26a and b).

The size of the new incision permits sufficient work space to drill into the mastoid, to carry out a posterior tympanotomy and cochleostomy (the orifice through which the electrode array is inserted into the cochlea) after securing the RS in the bony well made in the temporal bone.

The small size of the incision makes drilling the bed of the R/S more difficult. The maneuver is done in poor lighting and in a cramped space under a small subperiosteal pocket and extends the duration of the surgery by10-25 minutes depending on the surgeon.
Our retractor with a light source, would create a more spacious surgery site and offer better lighting conditions which are necessary for drilling the bone under the flap to create a bed for the R/S. This greatly shortens the number of maneuvers needed and the time allocated for such an operation.

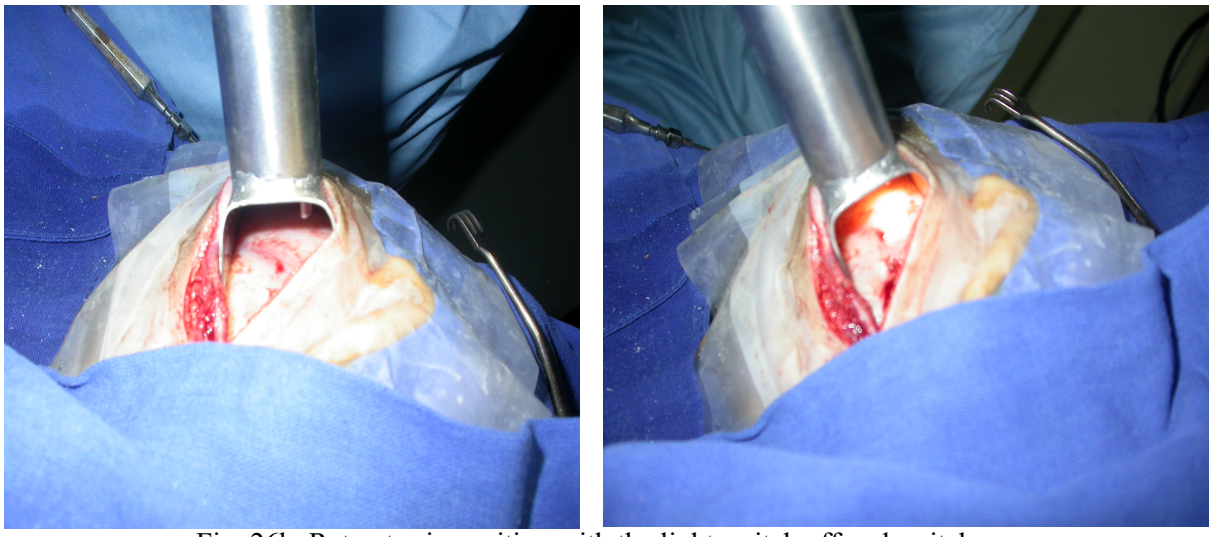

Fig. 26b Retractor in position with the light switch off and switch on.

The footplate of the retractor is introduced through the small retroauricular incision underneath the subperiosteal flap for a distance of 40 mm (this is the length that corresponds to the size of the R/S) (Fig. 26 a and b). The footplate elevates the soft tissue that covers the bone creating an accessible space for drilling.

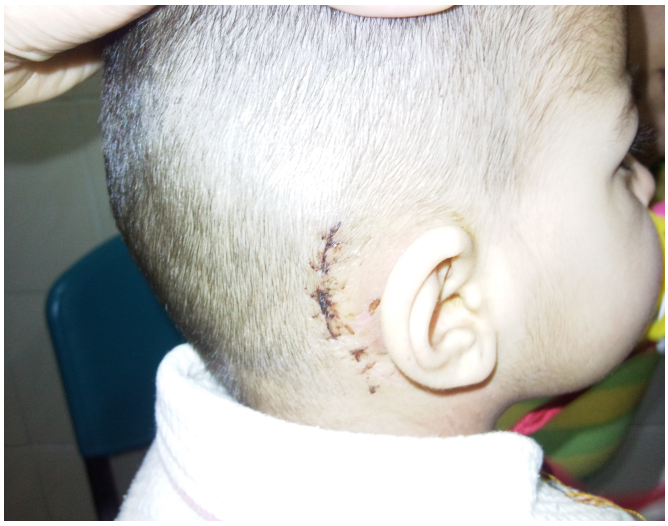

Fig. 27b The resulting incision through which cochlear implantation was performed using our new device

After drilling the bony bed, the R/S is accommodating, and the incision is sutured in two layers. A sterile and slightly compressive dressing is placed over the surgical wound.

Final remarks

The retractor is used in a difficult step of the surgical intervention.
The difficulty is that in a relatively small space located away from the incision site, a space in which the receiver/stimulator of the implant is placed without reaching the dura must be drilled in the external part of the occipital bone. The fact that the operating area is at distance from the incision, implies additional difficulties related to the resistance of the skin which must be sufficiently raised to allow access to the drill.

Moreover, the fact that the bed for the receiver/stimulator is made relatively far away under the skin implies a poor illumination of the surgical site.

The retractor solves these problems aside by raising enough the skin and at the same time perfectly illuminating the operating area and thus creates a sufficiently large space (wider than the classical technique that uses 2 elevators that are held by the assistant operator). At the same time, the strong lighting prevents the surgeon from reaching the dura-mater or other fragile structure more difficult to observe in the classical technique.

The advantages of this device from a surgical standpoint is the illumination system and the space the retractor creates making it possible to free up the surgeon's hands. The results of using this device are: a shorter operating time by about 15% and safer operating conditions - which are important especially in patients younger than 12 months of age.

This device helps us to the drill the bony bed for R/S through a small retroauricular incision. The advantages of this incision are: a shorter healing time, a reduced risk of post-operative complications and a shorter hospitalization. Also, it is more acceptable aesthetically, the small scar being barely visible (Fig.27b) when we compare with the previous incision (Fig.28 and Fig.21b).

These advantages appear to be more obvious when performing on children, especially in those under 12 months of age (the age at which the cochlear implant intervention reaches the best results). In this case the intervention site is much smaller, and the thickness of the cranial plate is much thinner and obviously more fragile, which implies additional risks.

It is noteworthy to mention that this retractor also increases the precision of the surgeon who has to operate on a site which is maintained at the same size by the fact that when the elevators are held by the assistant operator there are differences in time in the manual traction which causes changes of the proportions of the operating site thus disturbing the surgeon.
In conclusion, we succeed to reduce the operating time and increase the surgeon's accuracy.

We also noticed that in the case of using the retractor the incision may be smaller.

Conclusion

The surgical intervention becomes more precise when a unique incision is made and when the surgeon has control over the traction. Also, the aesthetic results are far much better given the smaller incision that is made.

I.3.4 The danger of the biofilm - understanding biofilm formation

Background

Bacterial biofilm formation is considered to play a major role in the pathogenesis of biomaterial associated infections (BAI) (Anderson and Patel, 2013).

The use of medical devices (catheters, artificial heart valves, prosthetic joints, cochlear implants etc.), increased dramatically over the past century and has become a major part of modern medicine and our daily life. The risk for BAI may in part be explained by the reduced efficacy of the local immune defense induced by the foreign body. It was shown that the number of bacteria required to cause an infection is significantly lower in the presence of a foreign body, such as in case of implants than when such devices are not present (Van Epps and Younger, 2016).

Cochlear implants are considered nowadays standard of care for patients (children and adults) with profound sensorineural hearing loss.

There were an estimated 34–36 million adults with measurable hearing loss in the United States in 2009 (Kochkin, 2009). Of that number, 1.2 million children and adults (with severe to profound hearing loss) were thought to be potential implant candidates (iData, 2010). The total number of CI recipients in the United States in 2009 was estimated at 70 000 adults and children (iData, 2010) and around 500.000 worldwide.

The development of infectious complications after cochlear implantation is rare, with an incidence of 1% to 4%. (Johnson et al., 2007)

The precise mechanism of infection is unknown.

Cochlear implants (CIs) may be exposed to bacteria not only at the time of the surgery but also thereafter from an infected middle ear. It was already shown the possibility of inner ear contamination directly from middle ear or by hematogenic dissemination in cochlear implanted patients and from here to meninges (Cohen et al., 2005). Meningitis being more frequent in CI recipients than in general population, both routes of infection have been investigated in animal studies (Wei et al., 2006; Wei et al., 2006).

We presume that the same mechanism may happened when infection from the middle ear is spreading through R/S causing BF formation on it, followed by device or flap failure and imposing explantation. This was reported in literature in 1 to 4% of cases (Klein, 1989).

It is generally assumed that middle ear is sterile in healthy individuals.

Infectious disorders (including acute otitis media (AOM), chronic (COM) and otitis media with effusion (OME) are quite frequent in children and, more than this, the microbiological status of the middle ear after the healing process it was not assessed. It is not known if a biofilm is formed in the middle ear cavities.

It is thought that between 50% and 85% of children experience at least one episode of AOM by 3 years of age with the peak incidence being between 6 and 15 months (Johnson, 2007).

OME can affect as many as 80% of children at some stage, (Kubba et al., 2000; Van Zon et al., 2012) with approximately 2.2 million new cases of OME annually in the United States of America. (AAFP, 2004)

Otitis may develop before or after implantation and theoretically carry a risk of CI contamination because of the possibility of bacterial persistence in the middle ear or on the skin in form of complex communities of sessile microorganisms. The BF can spread along the electrode array toward the R/S. OM is the most common bacterial infection during childhood so, children are especially put at risk. Most common microorganisms in AOM are Streptococcus pneumoniae and Haemophilus influenzae (Soriano et al., 2000).

It is also known that COM is a biofilm disease (Post et al., 2004).

Children with OME presented higher prevalence of S. pneumonia, M. catarrhalis and Staphylococcus in middle ear when compared to controls (Chan et al., 2017).

Biofilm formation allows microbes to survive in a hostile environment. Biofilms can be characterized as complex communities of sessile microorganisms embedded in a self-produced matrix, adhered to mucosal surface or an inorganic substrate. The main characteristic of BFs is their resistance to the defense (Otto, 2009; Chen et al., 2013) mechanisms of the host and to antibiotics, resulting in chronic infections. They also release planktonic bacteria causing recurrent infections. Direct evidence for the hypothesis that OM may be a biofilm disease was provided by the demonstration of characteristic matrix-enclosed adherent clusters of bacteria on the middle ear mucosa of children with COM who had not responded to multiple courses of antibiotics (Jensen et al., 2017).

Bacteria gain access to the tympanic cavity from the nasopharynx (which is naturally colonized by BF (Gilley and Orihuela, 2014) via the eustachian tube. In specific condition microorganisms adhere to the mucosal lining of the middle ear followed by a cascade of events that will end in BF formation (Davey and O'Toole, 2000; Post et al., 2004).

Personal contribution

Scientific and professional achievements:

Within this direction of study we made a review of the scientific literature concerning bacterial biofilm formation on cochlear implants. The review is important because it is the basis of the research project that we will start in September this year. We have published the review, in an ISI journal.

Published paper:
Georgescu M, Vrinceanu D, **Rădulescu L et al.** Microbial biofilms and implantable hearing aids. Romanian Biotechnological Letters 2017;22(4), 12681-12687.

Biofilms in middle ear infections

Biofilms represent a community of microorganisms adhered to an inert or cellular surface, and encased in an extrapolysacharidic matrix (Kania and Ars, 2015). Bacteria adhered to a surface and subsequent produced a "faintly staining" film which "increases with age" (Zobell, 1943). Unfortunately, antibodies are active just on planktonic bacteria and less able to control chronic and recurrent infections provoked by biofilms. Biofilm is very difficult to

remove either from the organism or from the implanted medical devices, so regaining the healthy condition of the affected area or the proper functionality of the prostheses is very challenging and often impossible without surgical removal and exchange of the medical device. Biofilms have many pores, acting as water channels (Flemming and Wingender, 2010). Through them, nutrients and oxygen are brought into the biofilm and metabolic waste is excreted from the biofilm) (Stoodley, 2002).

According with O'Toole and colleagues there are several steps in formation and development of the biofilm:

1. Adhesion – bacteria bind to organic and inorganic surfaces, initially in a reversible mode. Biofilm formation depends on the temperature, osmolarity, pH, iron, oxygen of the environment and also depends by bacterial species included. Bacteria prefer areas with high shear stress, where the cells are pressed against surfaces. Biofilm formation is a dynamic process in response to the environment, is a quick process – it can take place in few hours.
2. Colonization and microcolonies formation – intercellular adhesion appears, as starting point of the biofilm. This reversible phase of biofilm formation is based on cell-cell interactions, probably mediated by intercellular adhesions. Cells start to generate large quantity of extracellular polymeric substances: polysaccharides, proteins, nucleic acids, and phospholipids that will form the biofilm matrix.
3. Maturation – depending on communication signals able to regulate distribution of the individual bacterial species, protein expression in surrounding cells and gene expression inside the biofilm. Thus, bacteria in the biofilm replicate and reach the concentration where they begin to regulate virulence and pathogenicity genes.

Each cell in the biofilm has the ability to differentiate in a protected phenotype status which can tolerate the antibiotics. Additionally, repetitive exposure to antibiotics modifies the biofilms, leading to increased resistance to antibiotics. Biofilms also exhibit resistance to ultraviolet light. High resistance of biofilms to antibiotic, chemical or physical treatments is based on the physical barrier created by the bacterial overcrowded, low replication rate of the internal layers (metabolically passive) and hard, continuously reshaping external barrier created by polymeric substances. Biofilm's own barrier blocks antibiotic diffusion into the biofilm, and also blocks the effects of immunoglobulins, superoxides and opsonines (antibodies bind with bacteria which facilitates white blood cell phagocytosis). Maturation converts microcolonies to large structures, resistant to host immune defenses, antibiotic and industrial sterilizing substances.

4. Dispersion and detachment. In this last stage, bacterial cells detached from the biofilm structure and could disseminate and colonize other surfaces or tissues from the hosts. Environmental changes as nutrient starvation or availability triggers this process (O'Toole et al., 2000).

Bacterial infection in the middle ear space can occur. Natural open communication between middle ear cavity and nose/nasopharynx, the Eustachian tube, increases the risk of middle ear infection (otitis media), especially in children, where the auditory tube is more horizontal than in adults, favoring transudate stagnation in the middle ear. Besides the acute episode of infection when free swimming (planktonic) bacteria replicate and proliferate rapidly, bacteria can also form minicolonies/biofilms in the middle ear cavity and Eustachian tube. In inflammatory or infectious diseases in middle ear mucosa exudate is present. Any surface in frequent or permanent water-contact will be covered by a biofilm. This condition by itself favors chronic and recurrent otitis media and biofilm formation. For these reasons, middle ear pathology and surgery have a high risk of biofilm pathology and specific protocols should be taken into consideration. Persistent, chronic, or recurrent otitis media can affect hearing

structures by themselves, and also the implanted hearing aids (prostheses) due to the biofilm coverage. Complex biofilm's structure modifies the characteristics and properties of the affected surface of the prostheses, leading to device failure. For this reason, any risk factor of the infection is taken into account before and during surgery. The cells from biofilm are dormant cells, metabolically inactive, which makes them resistant to antibiotics and biocide. Whitely and al. showed that even if a bacterial strain is sensible to an antibiotic, the same bacterial strain grown in biofilm may be highly resistant (Whiteley et al., 2002). For this reason, "sterile" surgery of the middle ear is mandatory and also antibiotics are recommended intraoperatively and shortly in postoperative period, in order to eliminate any bacterial contamination and leave no bacteria in place.

Type of implantable hearing aids

Depending on the type and severity of the hearing loss, various rehabilitation methods are available, including a large variety of implantable hearing aids. All of them have a surgical-implantable component to be placed in the mastoid bone, middle or inner ear: i) bone conduction hearing aids are recommended for specific conductive hearing loss– they are surgical placed and fixated into the mastoid bone. Thus, they transmit directly to the cochlea the vibration of the sound by skull's vibration; ii) middle ear implants can be used in moderate sensorineural hearing loss– it is fixated either through one of the ossicles of the middle ear, either on the round window, thus transmitting the vibration of the sounds to the inner ear fluids and stimulating the hearing sensorial cells; iii) cochlear implant represents the only appropriate treatment option for severe to profound hearing loss– they are surgically placed directly into the damaged inner ear and stimulate the auditory nerve.

Implantable hearing aids are designed for life-time use and technology continuously develops to accomplish this goal, mainly addressing to biocompatibility issue.

Prevention of biofilm formation is linked to the prosthesis manufacturing process and also to medical protocol (surgery associated with perioperative antibiotic prevention). Surface properties of the material are very important – texture, nanostructure and porosity significantly influence the infection risk (Desrousseau et al., 2013). Smoother implant surface, with no dead-spaces (Zimmerli, 1993) are the best choice to avoid bacterial adhesion. New approaches to biofilm prevention include anti-microbial coating of the implantable hearing-aid.

From medical point of view, surgery on a non-infected middle ear is preferred. Low-bleeding procedures are recommended, since proteins from blood and transudates induced by increased vascular permeability and inflammation, infection or trauma transforms the prostheses' surface in a more permissive one for bacterial adhesion (Anderson, 1993).

Cochlear implants

Cochlear implants are, maybe, the most frequent topic for biofilm research since they are used mainly in small children (1 to 4 years old) and they should be functional for their entire life. Biofilm formation has a high-risk formation in cochlear implanted children because during childhood recurrent otitis media is frequently met (75-80% of children under 3 years age had at least one episode of otitis media (Grevers, 2010).

Haemophilus influenzae as well as Pseudomonas aeruginosa and Staphylococcus aureus, main etiologic agents of otitis media, are well known as components of upper respiratory tract and middle ear biofilms (Soriano F, 2000).

Anatomical particularity of the Eustachian tube in children, previously discussed, explains the high incidence of otitis media in small children. These predisposing conditions in children are an additional argument for rigorous medical protocols prior and during cochlear implantation. Some precautious are mandatory in cochlear implant (CI) surgery - internal part of the cochlear implant should be placed in a proper position, on smooth skull surface, flap have

to be closed tight, sterile sutures are used to avoid stitch abscess and H_2O_2 dressing is used to destroy preoperatively possible biofilms.

Major infectious complications (3% of cases) are acute suppurative otitis media or mastoiditis, surgical wound (flap) infection (dehiscence or necrosis), retroauricular abscess or meningitis.

Postoperative infection is rare in cochlear implant surgery (1.7 to 3.3%) (Cunningham et al., 2004). Usually, skin-flap infection is the origin of persistent infection which may lead to biofilm formation on the hearing prostheses and secondary surgery for explantation of the infected hearing aid. Also, head trauma, very frequently met in children, can lead to cranial hematoma in the vicinity of the cochlear implant. Hematoma has a high risk of bacterial infection and biofilm formation due to the high protein concentration which leads to bacterial adhesion.

Chronic middle ear infections are caused by Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus pyogenes and mastoiditis by Streptococcus pneumoniae. Delayed infection usually appears due to Pseudomonas aeruginosa infection. Middle ear infections and meningitis are more frequently met in patients with chronic ear disease (Cunningham et al., 2004).

In order to prevent these severe complications, CI protocols include active immunization against Streptococcus pneumoniae and Haemophilus influenzae type B, combined with perioperative antibiotic prophylaxis.

Flap infections have another pathogenic mechanism, skin contaminant at the time of surgery (Darouiche, 2003), since the most common pathogen found in CI patients with skin infection is Staphylococcus aureus and epidermidis (Dancer, 2008).

Methicillin-resistant Staphylococcus aureus (MRSA) is resistant to multiple antibiotics and explains persistent infection in CI patients. This organism, as well as Pseudomonas frequently causes biofilms on implants, which may reduce the susceptibility of microorganisms to antibiotics. High virulence of the MRSA infection was explained by specific molecular changes detected in MRSA infection, such as quorum sensing, adhesion, bacteriophage mobilization, exotoxin production, intracellular persistence, and biofilm formation (Otto, 2008). These particularities lead to a potential synergy between biofilm formation and MRSA infection, with severe sequelae in CI patients. Inappropriate antibiotic use increases MRSA infection rate, as well as its pathogenicity.

Implant's surface, with its irregularities, plays an essential role in the establishment of bacterial biofilms. Once host matrix proteins cover the abiotic surface of the CI, biofilms can grow and mature much faster (Jang et al., 2010). Together to sterile surgery and incision care, further preventative measures should be taken, including coating devices or magnets with antibiotics or an inert material (Pawlowski et al., 2005).

Clinical studies regarding complications of CI surgery demonstrated substantial presence of biofilms in depressions mainly on the magnet part and less common on the CI array. This comes not from a perfectly smooth surface of the electrode-array, but from tight seal of the cochleostomy or round window entrance of the array in the cochlea (Loeffler et al., 2005; Im et al., 2015).

The central region of the magnet is the most exposed part and biofilms are thickest at this level than in the periphery of the internal part, due to its proximity to the periosteal pocket.

Complex three-dimensional structures with water channels in the middle region were observed under high magnification, providing optimal conditions for bacterial growth. Mature biofilm forms a plate-like barrier by its outer wall, a unique pattern of over- maturation, because biofilm on a CI grows in a confined space and cannot detach or disseminate (Lewis, 2001).

Biofilms are a possible cause of implant failure, especially after MRSA infection. Failure comes by different ways: i) biofilms protect bacteria from antibiotics, leading to a latent

and persistent infection; adverse effects of biofilms include facilitation of bacterial metabolism and growth, blockage of antibiotic penetration, and increased resistance to antibiotics; ii) biofilms might induce an allergic reaction, with secondary persistent inflammation (Langelaar, 2010).

Unfortunately, persistent infection of a CI leads to surgical removal of the infected biofilm covered internal part of the cochlear implant. The electrode array, usually spared by the biofilm formation, is left in place in the scala tympani for second stage re-implantation, in order to prevent cochlear ossification, even in cases of MRSA infection.

Conclusions

Auditory rehabilitation by mean of implantable hearing aids requires minimal risk factors for developing middle ear infections. Once formed, biofilm leads to hearing prostheses' failure, by maintaining chronic infection and deteriorating the characteristics and properties of the materials of the hearing aid. This complication should be avoided especially in cochlear implanted patients, since its replacement implies a more complex surgery than for other hearing prostheses.

Besides the surgical aspect, biofilm from the affected components (mainly the magnet, not the electrode) must be completely removed before opening the cochlea in order to avoid secondary meningitis. Due to high antibiotic resistance of the biofilms, the clearance of infection is a major challenge and needs further research. Antimicrobial coating of the implantable hearing aids seems promising for biofilm formation avoidance.

I.3.5 The role of nanoparticles in deafness treatment

Background

On demand drug delivery at disease-specific site with minimum side effects represents a great challenge for nanomedicine, hence the efforts are now focused on designing multi-functional nanocarriers to release drugs (nanoparticles) at the particular location.

In case of congenital hearing loss the speech development is affected with an important impact on academic achievements and further on in adulthood when might play a role on restricted career choice and possible lower earnings (Schroeder et al., 2006). Furthermore, in the adult, the hearing loss leads to social isolation and behavioral disturbance up to depression. The consequences of deafness may be devastating.

The prevalence of hearing loss was first assessed in 1985. At that time it was appreciated that approximately 42 million (0,9%) of people of the world population have disabling hearing impairment (Smith, 2003). Disabling Hearing Loss (DHL) is defined as the loss of hearing greater than 40dB in the better hearing ear in adults and greater than 30dB in the better ear in children. The estimation made 25 years later shows that 360 million (5,3%) people worldwide have DHL**. Of these, approximately 32 million (9%) were children, 7,5 million being younger than 5 years ***. The escalation of DHL prevalence starting from 1985 until 2011 has several explanations. Starting from the year 1978 the problem of hearing loss screening was emerged at a large scale. The Saskatoon Conference on Early Diagnosis of Hearing Loss recommended in 1978 the registration of neonates as being at high risk for hearing impairment, a resolution requesting provincial and local governments to make registration mandatory was passed with that occasion (Gerber and Mencher, 1978).

On the other end are the elderly. The rise of mean life expectancy from many countries leads to increased prevalence of presbyacusis and not at least the increasing exposure to the. The occupational and environmental noise might be also an important cause of hearing impairment. To these 3 factors that explain the rise of prevalence of hearing loss there has to

be added the fact that none of the previously known factors to cause hearing loss has been removed, these factors still contributing in hearing loss. These classic factors that lead to hearing loss development are: ototoxic drugs (more important the aminoglicosid antibiotics and citostatic drugs) – so useful in treatment of serious illnesses, the infectious – especially viral ones – but not only: urlian virus – responsible for mumps, rubella virus, etc. In this field, important progress has been made regarding the prophylaxis (Seidman et al., 1997; Gao et al., 2017). Genetic factors are responsible for more than 50% of congenital deafness (Gasparini et al., 20000. Although real progress has been made in detecting gene mutations responsible for deafness, the development of a prophylactic and/or curative treatment is still on the wish list. Sensorineural hearing loss is nowadays one of the most common diseases accounting for about 50% of all disabling diseases (Li et al., 2016).

Personal contribution

Scientific and professional achievements:

Regarding this subject, we have made an up to date of the scientific literature concerning the use of nanoparticles (Nps) in the pathology of the cochlea. Np might improve drug delivery to the inner ear facilitating crossing the blood labyrinth barrier and targeting more specifically the inner ear. The review is important because it was the basis of an application to an European call early this year. We have published the review, in an ISI journal.

Published paper:
Martu C, Georgescu MG, Martu I, Butnaru C, Porumb V, **Rădulescu L**. Utility of Drug Loaded Nanoparticles in the Treatment of Inner Ear Pathology. Materiale plastice. 2016; 53(2): 321-325. (IF = 1,248)

Treatment of sensorineural hearing loss

Treatment for sensorineural hearing loss (SNHL) has evolved from systemic administration of drugs (ex. Ototoxic drugs for intractable Meniere disease) to local application of pharmaceutical agents. The systemic administration of medications was started in the middle of the 20th century when Fowler 1948 gave streptomycin to his patients for control of vertigo (Fowler, 1948). After more than half of a century systemic administration of ototoxic drugs (streptomycin) continues to be possible in the treatment of bilateral Meniere's disease by intramuscular drug delivery and the dose calculated by the clinical effect (Berryhill and Graham, 2001). Among the systemic use drugs there are: steroids as standard treatment in sudden deafness, alone or combined with antiviral drugs (Plontke et al., 2016) – although there are authors that question the efficacy of systemic steroid use (Conlin and Parens, 2007) – diuretics used in Meniere's disease treatment (Coelho and Lalwani, 2008), biphosponates in otosclerosis (Quesnel et al., 2012), vasodilators intravenous in sudden deafness and tinnitus treatment (Probst et al., 1992), Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss (Kaya et al., 2015) and Betahistine (Novotny and Kostrica, 2002) (H1 receptor agonist and H3 receptor antagonist) or Arlevert (Scholtz et al., 2012) (combination of cinnaryzine -and a calcium channel blocker and dimenhydrinate –an inverse agonist of the histamine a H1 receptor) given oraly are used in the chronic treatment of Meniere's disease, in vertebrobasilar insufficiency or vestibular neuritis.

All above mentioned therapies are in current clinical use although they have significant limitations. The main concern regarding systemic administration is represented by the potential undesirable side effects that may range from minor to life threatening effects – like in, but not

limited to, systemic steroid therapy (Faucher et al., 2008). The second important limitation of systemic route for inner ear delivery of medication is the highly variable pharmacokinetic profiles that result in variability in concentrations of the drug at the level of the cochlea (Plontke et al., 2002). This variability is determined by the patient (age, renal and liver problems, genetic predisposition). Regarding the anatomical condition of the targeted organ – inner ear in our case – it is important to emphasis the anatomic inaccessibility of the cochlea due to the blood-cochlear barrier (Swan et al., 2008).

The round window approaches

To overcome blood-cochlear barrier, the round window (RW) approach was proposed. Although adverse effects associated with systemic administration are eliminated, the transtympanic administration raises some problems. The most important seems to be the necessity of repeated administration – even in the same day (Salt and Plontke, 2009) because of drug loss through the Eustachian Tube (ET) and different diffusion rate of the drug through the RW membrane according to the individual structure and thickness (Salt and Plontke, 2009).

Administration of different drugs like steroids (Ng et al., 2015) or insulin like grow factor type I (IGF I) (Nakagawa et al., 2014) through RW to treat sudden hearing loss or neurotrophic factors to restore synaptic connection and to promote neuron outgrowth after noise trauma show promise (Glueckert et al., 2008), but specific delivery methods of drugs towards the inner ear are necessary.

One other problem consists in the lack of the specificity of the drug for the sensorineural structures. In this regard, the European Union Consortium (NanoEar) developed some nanoparticles (Np) for specific targeting the sensorineural structures from the inner ear. The specific organ targeting is possible using the tropomyosin receptor kinase B (TRK B) which is a specific receptor for brain- derived neurotrophic factor (BDNF) (Glueckert et al., 2015).

Nanoparticles for specific targeting the sensorineural structures from the inner ear

Nps are particles of very small size, with nanometric range dimensions. General, the term of Np is referring to particles with the size ranging from 1 to 100 nm. The structure of a Np has to be constructed according to the function that they have to accomplish. To fulfill a certain function, the size and the shape of the Np is of major importance.

Nps have different properties from the bulk solid of the same material. The changes of the characteristics are due to the small size of the Np – and this phenomenon is known as size effect. There are two reasons for these differences. First, the molecules located at the surface of the Np are more active because of the free hand and thus they can bond easier with different materials. Secondly, the specific surface area in a Np is increasing in reversal proportion to the particle size and eventually they can load and carry more active substance to the diseased target. In order to use Np for medical purpose they have to disassemble in the components that will be utilized by the human body. The most studies regarding the use of Nps in otorhinolaryngology are related to the inner ear diseases. The interest in the use of Np in the pathology of the cochlea derives from the necessity of a targeted therapy at this level. Np might improve drug delivery to the inner ear facilitating crossing the blood labyrinth barrier and targeting more specifically the inner ear. There are Nps described to cross the RW membrane (Liu et al., 2013) to reach the sensory cells in the cochlea. Typical the size of Nps used for drug delivery to the inner ear is in the range of 200nm or less (McCall et al., 2010). The RW membrane in humans consists of three main layers (epithelial, connective tissue and cellular), with a variable thickness across different species (Nordang et al., 2003) that behaves as a semi permeable membrane, protecting against substances washing out from the inner ear towards the middle ear (Goycoolea, 2001). Furthermore, the permeability of the RW membrane is also variable, depending on what vehicle or method is used to cross it (Goycoolea, 2001). For example, the permeability is increased by

local anesthetics like tetracaine (Hoft, 1969) or by histamine (Chandrasekhar, 2000) or by lowering the humidity at the level of the RW niche (Mikulec et al., 2008). Once the drugs are passing the RW membrane, the presence of a basal- apical gradient in the Scala tympany was reported by numerous studies, with a higher concentration in the basal turn than in the apex (Zou et al., 2015). These differences are due to the slow rate of the perilymph and endolymph flow inside the cochlea and also to the loss of drugs to the adjacent compartments (Shepherd and Colreavy, 2004). Crossing the RWM might be done by passive diffusion and also by active targeting mechanisms using some proprieties of the Np (ex. magnetically guided NPs in drug delivery for the inner ear application shows promise (Du et al., 2013) or ligands directed against receptors of the surface of targeted cells (Schubertova et al., 2015).

On demand drug delivery at disease-specific site with minimum side effects represents a great challenge for nanomedicine, hence the efforts are now focused on designing multi-functional nanocarriers to release drugs at the particular location – referred as NanoCure (Bourzac, 2012). To facilitate enhanced drug delivery from the Np, some methods of stimulation have been proposed: thermal, optical, electrical or acoustic activation (Sagar et al., 2014).

Poly-lactic glycolic acid nanoparticles

Biodegradable as well as non-biodegradable Nps were investigated for inner ear drug delivery purposes. The best-known biodegradable particles are those generated from poly-lactic glycolic acid (PLGA). PLGA was approved for human use by the US FDA and has been established as the best NP drug carrier (Di Toro et al., 2004). PLGA administered at the level of the RW was found in high concentration in the cochlea of chinchilla showing good capabilities for delivery of drugs to the inner ear (Ge et al., 2007). The next step was to demonstrate that Dex-Ac has a good loading in PLGA (Wang et al., 2011).

In 2015 Sun C have demonstrated that PEG-PLA Np loaded with Dexamethasone has a hearing protection effect against cisplatin induced deafness (Sun et al., 2015). The authors have applied PEG-PLA-Np (labeled with coumarin 6 – a fluorescent labeling dye) and also free cumarin into RWM in guinea pigs through a bulla opening to determine the distribution of Nps in the cochlea after RWM delivery. They found that in guinea pigs treated with Nps labeled with dye the fluorescence was strong in all cochlear turns and also in modiolus compared with free administration of the dye when the fluorescence in the cochlea was weak. In the second experiment they have compared the concentration of drug in the cochlea after free Dexamethasone vs Dexamethasone Nps administration through the RWM of the guinea pigs. They found that the concentration of free Dexamethasone was significantly lower than that of Dexamethasone Nps. When otoprotective effects of Dexamethasone Nps were evaluated, a significant attenuation of cisplatin-induced hearing loss in guinea pigs was found, proven by both functional and histological evaluation (Sun et al., 2015).

The final aim was to prove that targeted delivery of a drug (dexamethasone) into the inner ear through RWM is possible in mammalian using PLGA magnetite nanoparticles under an external magnetic field. It was demonstrated that in association with PLGA and magnetite and in the presence of an external magnetic field Dexamethasone Acetate reaches significant higher concentrations in cochlea when compared to passive diffusion (Du et al., 2015).

Amorphous silica-based Np

Some other particles that have shown promise for inner ear delivery are silica Nps. Amorphous silica-based Np are a new class of mesoporous materials with a large surface area and pour volume for high drug loading. The particles are biodegradable and biocompatible. Placed against RWM of mice, silica Nps were found in the cochlea without any toxic effect on inner hair cells or vestibular hair cells (Praetorius et al., 2007). Silica particles can be

functionalized with different surface modification. Glueckert et al. had tested silica Nps with surface modification by peptides mimicking tropomyosin receptor kinase (TrkB) activating antibodies. They found that agonistic antibodies linked to silica nanoparticles showed TrKB activation and enhanced binding to inner ear cells (Glueckert et al., 2015).

Liposomes

Liposomes are the most common used Nps (Fenske and Cullis, 1993). They are phospholipid two-layer vesicles with the size in rage of 50 to 200nm. They can be composed of Sph, egg PC, DSPE- PEG-2000, peptide – PEG-lipidic conjugate and DPPRho. The main advantage of liposomes derives from their amphiphilic nature that allows them to carry both hydrophobic and hydrophilic molecules. Other important advantages of liposomes are: greater stability of large compounds, extended circulation lifetime when coated with PEG with prolonged systemic drug exposure and high cellular penetration (Fenske et al., 2002). New generations of liposomes are represented by: pH sensitive liposomes (used to deliver the drugs to intracellular target site), thermo sensitive liposomes – that require the addition of an external stimuli – hence their use might be limited to local treatment, magnetic liposomes (lipid coated nanomagnetic particles) – that also require external application of an magnetic field to attract the drug carrier to the diseased site. Multifunctional liposomes – combine several of all possible properties in a single nanosystem to finally facilitate the accumulation of drug to target site and to minimize side effects. Various side effects were reported for liposomes such as: different types of skin reactions – most common being hand-foot syndrome (Lotem et al., 2000) and hypersensitivity reaction like hypotension or hypertension, dyspnea, flushing, rash and feeling of chocking were found in 30.8% of patients treated with doxorubicin (Chan et al., 2007). Liposome reactions in such patients can be life threatening (Szebeni et al., 2005). Jing Zou et al in 2015 validate a delivery system of liposomes for inner ear therapy. They found that liposome nanocarriers containing gadolinium tetra-azacyclo-dodecane-tetra- acetic acid enters in the cochlea through both oval and round window reaching therapeutic doses (Zou et al., 2015). In 2013 Okada et al found that liposome encapsulated hemoglobin (an artificial oxygen carrier) might be utilized in preventing hearing loss due to cochlear ischemia (Okada et al., 2013). In 2016 Kechai shows that local administration of corticosteroid embodied in hyaluronic acid liposomal gel is efficient for sustained drug delivery to the inner ear (Kechai et al., 2016). Delivery of DNA in the inner ear using liposome was also subject of evaluation. One study conducted by Wareing demonstrates the potential of liposome as drug carriers for gene therapy (Wareing et al., 1999).

Dendrimers

Dendrimers are water soluble tree-like branched polymers. At the end of their branches are located functional groups that can have positive, negative and neutral charge. Poliamidoamine (PAMAM) dendrimers have ideal properties for gene delivery: high positive charge, a high flexible structure, an internal cavity surface shell, fast biodegradation (Tomalia et al., 1985).

Conclusions

Experimental research on animals provide proof of efficiency of Np in the treatment of deafness but further studies on human patients still need to be performed to achieve results towards better treatment options for inner ear pathology.

I.4. PERSONALIZED FITTING AND EVALUATION

I.4.1 Personalized fitting - Cochleo-vestibular reflex

Background
Objective measurement of hearing discomfort level – preliminary results

Hearing loss represents the most frequent disease in newborns. To avoid the consequences of deafness is necessary to diagnose and to treat it as soon as possible. The success with a hearing aid depends on the accuracy of the fitting process. The hearing threshold and the discomfort threshold are different from one patient to another, for each and every frequency, and for the cochlear implant, the stimulation values are different for each electrode. More than this, the fitting process may be repeated for several times because of the neural plasticity that leads to changes in the threshold values.

Traditionally, these thresholds are established by mean of behavioral methods. The difficulty with these techniques is related to the age of the child, obtaining the discomfort levels from young children may be extremely problematic. To avoid any possible errors is necessary to have an objective method to assist the clinician in the process of hearing aid fitting.

Personal contribution

Scientific and professional achievements:

Within this direction of study I design a pair of glasses that could record the movement of the eyelids. With this glasses I have studied the cochleo-palpebral reflex in normal hearing people related to discomfort levels of noise. We published a paper in a Suppliment of Rev Med Chir Soc Med Nat . The method might be useful in fitting the CI in infants.

Published paper:
Rădulescu L, Curcă AI, Butnaru C, Mârţu CM, Mârţu D. Cochleo-palpebral reflex as an objective measurement of discomfort level in healthy adults preliminary results. Rev Med Chir Soc Med Nat Iaşi. 2008; 112(1). ISSN: 0048-7848

Aim of the study

The aim of the study was to find an objective reliable method to assist the CI fitting in infants.

Patients and methods

The study included 22 healthy subjects: 16 females with a main age of 23 years old and 6 males with a main age also of 23 years old (all male subjects were 23 years old).

No otologic complains were noted from the history of subjects and all of them appeared normal to otoscopic evaluation.

A middle ear analyzer was used to perform tympanometry and to record stapedius reflex. Tympanometry was performed in all patient prior to stapedius reflex testing.

Behavioral hearing threshold and discomfort level were obtained on three frequencies: 500 Hz, 1000 Hz and 4000 Hz.

The cochlea-palpebral reflex was recorded using an EMG. The two surface electrodes were placed over the upper and respectively over the lower eyelids. The blink reflex related to pure tone stimulus was recorded for the same three frequencies (500, 1000, 4000 Hz).

Subject participation followed informed consent before inclusion in the study.

Results

The data obtained from measurement are presented in Figure 30 for females and in Figure 31 for males.

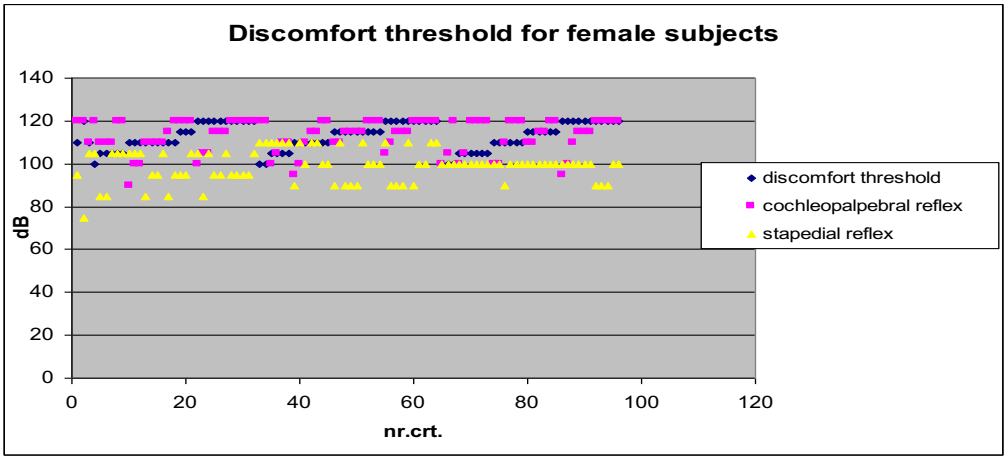

Fig.30 Results of discomfort threshold measurements in female subjects

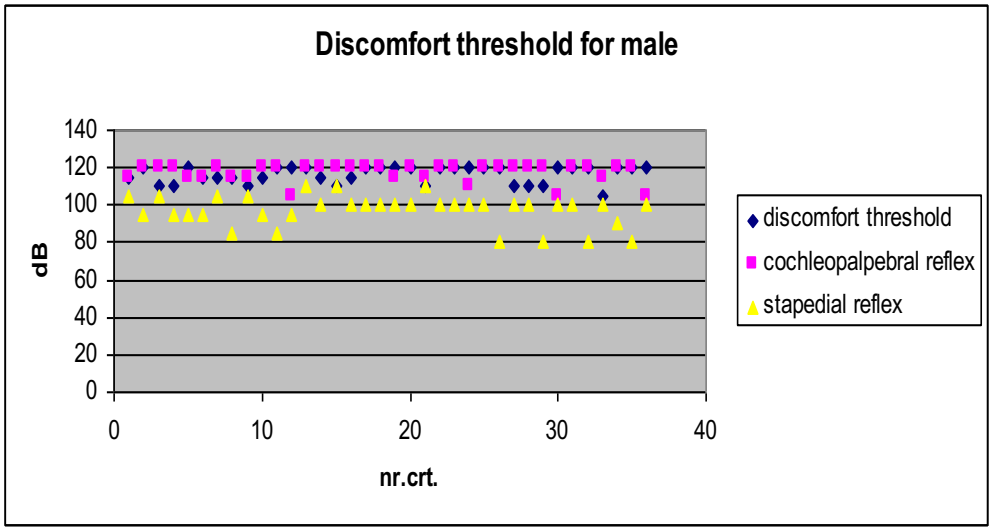

Fig.31 Results of discomfort threshold measurements in male subjects

Discussions

Being an original work – there are no bibliographic references even many years after our study

Analyzing the relationship between the values of stapedius reflex and the values of discomfort threshold for the three tested frequencies (500, 1000, 4000 Hz) we can affirm that

independent to the frequencies, on side evaluated the value of stapedius reflex is almost always below the values of discomfort threshold. In five cases the difference between the two thresholds stapedius reflex and discomfort threshold was 5 dB and in two cases was 10 dB, the stapedius reflex being above the discomfort threshold – all these cases were recorded at female subjects.

Regarding the relationship between the cochlea-palpebral reflex and discomfort threshold, in the majority of cases the cochlea-palpebral reflex was above the discomfort threshold but there was some situation when the cochlea-palpebral reflex was under discomfort threshold (15,6%). In most of these last cases the differences between cochleo-palpebral reflex and discomfort threshold do not exceed 15 dB (14 cases – 10,6%) and only in 1 case (0,75%) was 25dB, in 3 cases was 20 dB (2,27%)

Only in three cases (all in female subjects) – the cochlea-palpebral reflex had fallen below the stapedius reflex, the difference being 15 dB in 1 case and 10 dB in 2 cases.

The stapedius reflex was used in the past to establish the comfort threshold in cochlear implanted children intraoperatively (Battmer et al., 1990; Hodges, 1990; Jerger et al, 1988; Spivak and Chute, 1994). The lack of correlation between this reflex and the behavioral discomfort threshold (Jerger et al., 1986) as well as the fact that the stapedius reflex may be absent and is also time-consuming during surgery determined clinicians to renounce to it in programing cochlear implants.

The solution for a better indicator of discomfort threshold might be a poligraphic analysis consisting in determination of more than one type of measurement – stapedius reflex and cochlea-palpebral reflex.

In our study we found that the average between the two values might be a solution.

Conclusions

Further determinations are necessary to establish the conditions necessary for an accurate measurement of cochlea-palpebral reflex in terms of duration of stimulation, type of stimulus, etc.

I.4.2 Validation of questionnaires for outcome evaluation

4.2.1 LEESQ

Background

Recent studies show the necessity of the assessment of the auditive function; after being discharged from the maternity, even if the children successfully pass the hearing screening tests, because of the eventuality of late onset hearing loss.

The above presented LittlEARS1 Auditory Questionnaire is the first module of the LittlEARS1 test battery. The LittlEARS1 Auditory Questionnaire was developed and piloted to assess the auditory behavior of normal hearing children and hearing-impaired children who receive a cochlear implant or hearing aid prior to 24 months of age.

The Littlears Early Speech Production Questionnaire (LEESPQ) is focused on expressive-vocal behavior and assessment of the evolution of the auditory behavior of the normal hearing children. It evaluates especially pre-verbal speech production from the first 18 months of life, following reflexive behavior, pre-canonical vocalizations, canonical vocalizations, and post-canonical advanced forms.

From 1 to 6 months infants' speech production is characterized by pre-canonical vocalizations such as grunts.

From 6 to 10 months their speech production is characterized by canonical vocalizations, e.g. rhythmic production of duplicated: e.g.: ‚duh-duh','baba'or non-duplicated syllables: e.g.: 'madaba'.

From 10 to 18 months infants use post-canonical advanced forms, e.g.: closed CVC syllables, consonant clusters, jargon, and first words.

Personal contribution

Scientific and professional achievements:

Within this direction we participated in a multicenter study. The result consisted in validation in different language – and also in Romanian language a questionaire addressed to parents in order to use it in the following up the progresses in speech production of CI infants and small children. The resulted paper was published in an ISI journal

Published paper:
Coninx, F, Weichbold, V,Tsiakpini, L,**Rădulescu, L**.et al. Validation of the LittlEARS (R) Auditory Questionnaire in children with normal hearing International journal of pediatric otorhinolaryngology.2009,73(12): 1761-1768. Times Cited: 40.

Material and methods

The LEESPQ consists of 22 questions with YES /NO answers that assess hearing and speech development in children up to 18 months. The answers are based on parent's observation. Most questions include examples for a better understanding.

All those who answered to the questionnaire were fluent Romanian speakers.

The questionnaire was translated and adapted to the Romanian language.

We conducted two studies:

- First one was a cross-sectional study that included 106 normal hearing children aged 0-18 months.
- The second was a longitudinal study that includes 33 unilateral CI children with age at implantation lower than 3 years old.

Study I: Validation of the speech production questionnaire in normal hearing children cross sectional study

Criteria used for including children in the study were: age between 6 days and 18 months, normal hearing – (PASS the hearing screening in the maternity) and the absence of known disabilities or pathology. The sample with normal hearing children (106 subjects: 69 girls and 42 boys), who live in both urban and countryside areas, were divided according to their age in 18 groups.

The questionnaires were distributed to general practitioners in Iasi, Romania – and from them to parents. Each question had 2 possible answers, "Yes" and "No". The parents were instructed to answer "yes" – even if they noticed the respective behavior just once. We counted each "yes" answer (one yes being one point).

Study II Longitudinal study of speech production of ci children

Criteria used for including children in the second study were: age at implantation less than 3 years old and just one ear implanted. The SP questionnaires were distributed by two

members of the CI team from our clinic directly to the parents of CI children. The parents were instructed to answer "yes" – even if they only noticed the behavior once. We counted each "yes" (one yes being one point).

Results

We collected questionnaires from 33 parents. Out of these: 3 children have auditory neuropathy, 4 children have GJB2 gene mutations, 1 child was excluded from the study – has generalized congenital paralysis. Therefore, there were 32 children included in the study, out of which 18 were girls and 14 were boys.

The scores obtained are presented in the Figures 32 – 34 for both groups: normal hearing and cochlear implanted children.

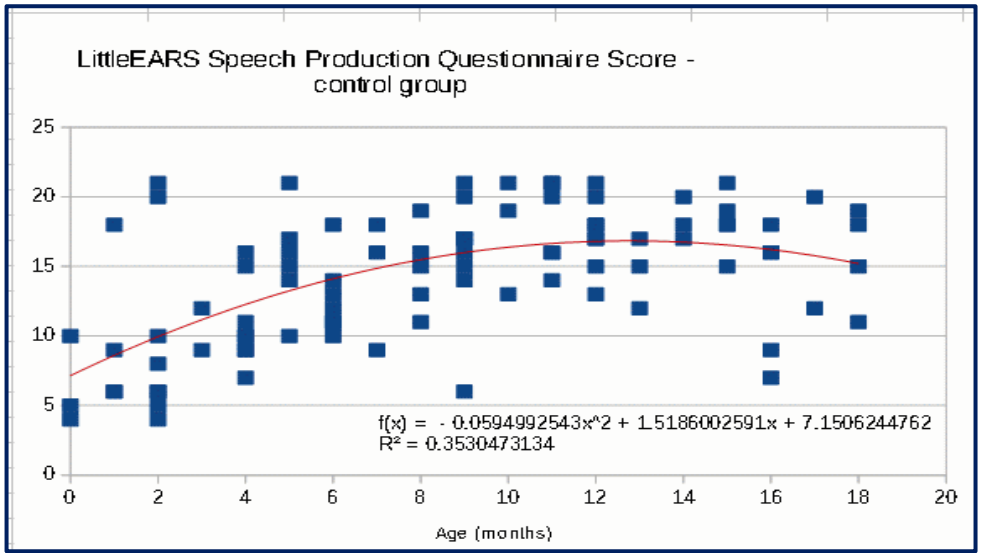

Fig. 32 SPQ Control Group Scores

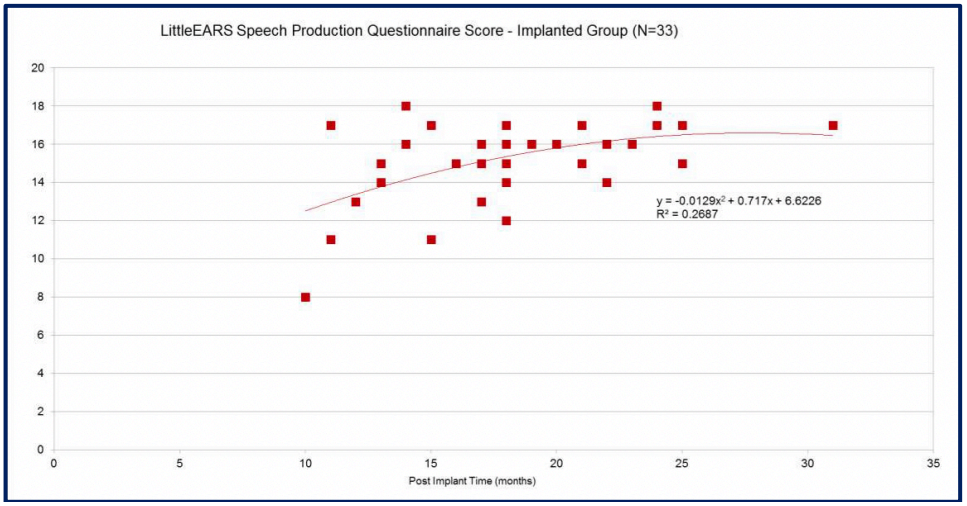

Fig. 33 SPQ Implanted group scores

Disscusions

Early identification and diagnosis of sensory neural hearing loss in toddlers and small children make possible early intervention (through hearing aids or/and cochlear implant).

The Speech Production Questionnaire (SPQ) might be a usefull tool to evaluate children's hearing behavior. It can be used as a follow-up method for children fitted with hearing aids or cochlear implants and as a screening tool for hearing in small children.

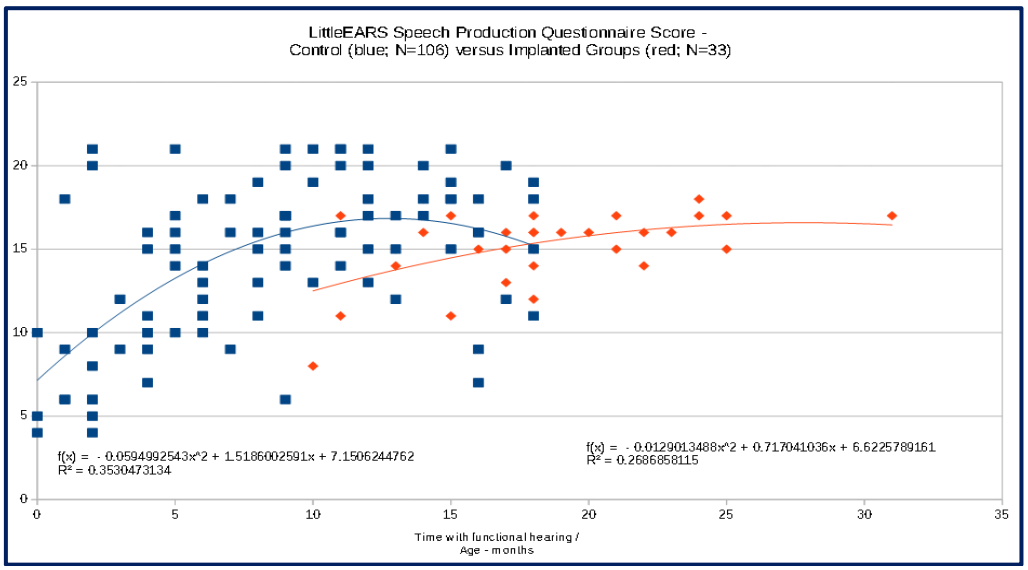

Fig. 34 SPQ Score-Control (blue) vs. Implanted Groups (red)

Study 1. Cross-sectional study for validation of spq in normal hearing children

The correlation between age and each question was calculated to select which questions are statistically representative/relevant for each age category. The determined value of *r* indicates a high and very significant correlation of SPQ with age.

We observed there is a statistically significant correlation between age and questions 1, 4, 7, 9, 10, 12, 13, 14, 15, 16, 17, 19, 20, and 21.

The present test gives an indication for the age dependent question. We found the minimum score that represent normal speech production for each age group. (Table 13)

The value for r^2 (0.3530) indicates a significant correlation of number of "yes" answers with the child's age of the minimum and expected values necessary for this version. (Fig. 32)

Study 2. Longitudinal study of speech production of CI children

After analyzing the answers gave by parents of CI children (Fig. 33), we noticed that their scores 10 months after implantation begin to be comparable with those of normal hearing children and equal with their score after 18 months from implantation (Fig. 34).

We noticed that only 1 in the 3 children with auditory neuropathy got a lower score compared to his age group. In the 14-15 months age group, the child who also has auditory neuropathy got the best score in the group.

Considering the value of r^2 (0.2687), we can affirm that the questionnaire is giving statistic significant results and is valid and suitable for toddlers with CI to assess their speech development after implantation.

Conclusions

Using the SPQ should contribute to increase parents' and educators' awareness regarding the importance of monitoring early speech evolution and language development.

4.2.2 Screening of auditory behavior in (normal hearing) children during the first two years of life

Background

Between 2005 and 2006 I was included in a large multicenter study. The aim of the study was **to validate" LittlEARS** Auditory Questionnaire" for **Romanian language** use. The questionnaire was developed to monitor the auditory behavior in small children.

- Universal newborn hearing screening program started in our region since 2008 offering the solution of early detection auditory disorders. An auditory **impairment acquired in pre-school children may go unnoticed** being detected late when specific effects appear vocabulary develops more slowly, the sentence structure is less complex, the speaking and diction are affected, (Kenna, 2015). Children with hearing loss or auditory processing problems continue to be an under identified and underserved social category. It has affirmed the fact that the earlier hearing loss occurs in a child's life, the more serious the effects on the child's development and the earlier the disorder is identified, and rehabilitation begun, the less serious the ultimate impact (ASLHA, 2015b). The delay in the development of receptive and expressive communication skills (speech and language) causes learning problems that result in reduced academic performance and the communication difficulties often lead to social isolation and poor self-esteem influencing vocational choices (Tomblin *et al.*, 2015).
- Since the development of pediatric cochlear implants, because the indications have expanded and the technology has made tremendous progress, expected outcomes in deaf children with cochlear implants are higher and higher. **The scales to measure their auditory capacities had to be modified** and reach now for some of them the level of hearing persons. Speech perception in children is a key element to monitor the benefit of this technique. It is generally assessed through scales like CAP (Category of Auditory Performance) and **questionnaires to families**. Speech perception starts rapidly after implantation by the detection of speech sounds and will improve to the ability, few years later, to understand a conversation on the phone even. Our study intended to fill this gap and to produce a useful tool in monitoring speech development of preschool child and of cochlear implanted one.

The LittlEARS Auditory was first validated in the German language in normal hearing children being foreseen to be used for:

- Longitudinal diagnoses after Universal – Newborn Hearing Screening
- Early assessment of possible hearing disorders
- Evaluation of the auditory development of hearing-impaired children after hearing aid or cochlear implantation.

The LittlEARS Auditory Questionnaire:

- is a parent questionnaire (refers to the daily observations made by parents)?
- includes 40 items which should be answered with yes or no.
- it needs around 10 minutes for the parents to fill it out.

Although the questionnaire is considerate to be language independent, as it covers mainly preverbal and early auditory development, there is no study which documents this suggestion.

To use the LittlEARS Auditory Questionnaire as a scientific evaluation method, there is a need for language specific norms on the one hand, or a documentation of the language independence of the outcomes of the other.

Material and methods

Participation in the study:

- Inclusion criteria - parents of normal hearing children of ROMANIAN (country of the language) in the age of 0 – 24 months.
- Exclusion criteria - unwillingness of the parent to cooperate

Subject identification - each subject who fulfils all inclusion and exclusion criteria and has signed the patient consent form is considered entered into the study. The patient is assigned a subject identification number according to the following scheme for normal hearing children: Country Abbreviation/ Town Abbreviation /Auditory Questionnaire Normal Hearing e.g.ROMISAQNH001; ROMISAQNH002;...; ROMISAQNHnnn. The subject identification number shall be used henceforth to identify the subject on all study related documents.

Test methodology

- Tests material - LittlEARS Auditory Questionnaire
- Parents from the sample group of normal hearing children will have to be acquired to fill out the questionnaire. These parents may be acquired from maternity wards or pediatric institutions. Another opportunity would be to search the New-Born Screening data base for children in the specific age.
- The parents have to sign a study agreement.

Sixteen different (languages) countries were participated in the study. From Romania there were included 88 children – 40 from our center.

The study was designed and conducted according to Declaration of Helsinki (1996) and MED-DEV 2.12.2 guidelines for conducting Post-Market Studies.

Adaptation and validation procedure

The stages involved in this process were: translation, validation of the translated English and versions, and development and validation of an overall norm curve.

The methods of translation and ‘‘back translation’’ were used for the first stage of the adaptation process. It was carried out by translators who were specialists in the field of audiology or speech-language pathology and experienced in translation. First, the LEAQ was directly translated from into Romanian by Translator A. A second translator (Translator B) then translated Translator A’s version back into English. In this way, the English translations from Translator B were compared to the English document originally used by Translator A. The discrepancies between the two English versions were discussed and settled so that the translated version was as accurate and true to the original as possible while still being culturally appropriate and measuring the same behaviors as the original version intended.

After completing the adaptation process, the LEAQ was given to a sample of parents of children with normal hearing. Data were used to determine whether scores obtained from the sample were valid and reliable. The same validation procedure used for the initial validation on the German speaking sample and described above was applied. In this way, a norm reference curve was obtained.

Finally, data from all children were used to establish an overall norm curve. The same validation process that was used for the initial German validation was also employed here.

Software - SPSS for Windows 12.0-16.0 software (Chicago, IL, http:// www.spss.com) and Microsoft1 Office Excel 2003 were used for all analyses and graphs.

Results

Scale analysis of language-specific data

A high correlation between age and score was found for every single language as well as for the overall sample. The coefficients lie between 0.80 and 0.93. Internal consistency,

calculated using Cronbach's alpha, is similar to the German study. In every sample as well as in the overall sample, Cronbach's alpha has a value greater than 0.7 (range: 0.93–0.98), meaning that the internal consistency is very good. The Spearman's split-half coefficient ranges between 0.88 and 0.96 in all samples. This indicates a high measuring accuracy. The same is also valid for the predictive accuracy of the scale, which is measured by Guttman's lambda. Values of Guttman's lambda lie between 0.85 and 0.94. There were no differences between total test scores of boys and girls in any of the samples ($p > 0.05$).

Comparability across languages and countries

A norm curve was calculated separately for every country. The aims of the establishment of norm curves were to have language-specific norms and to show language independency of the outcomes on the other hand. Pearson's correlation coefficients are in general very high. They range between 0.988 and 1.000. This implies very good comparability of all language-specific norm data, i.e., the German and Austrian curve and the overall norm curve.

Item analysis and standardized values of all data

Finally, data of all subjects who have participated in the study were subjected to an item analysis (the index of difficulty, the discriminatory power coefficient and the selectivity index were calculated for each item to assess the suitability of the items. The indices of difficulty range from 0.23 to 0.99. The discriminatory power coefficients in the overall sample range between 0.17 and 0.87. The same applies to the selectivity index. They range between 0.31 and 1.02. Standardized expected and minimum values are shown in Table XIII.

On average all data obtained was very similar with the German speaking sample.

Discussions

The results confirm that the LEAQ is a relevant, language-independent tool which may be used internationally for assessing the age-related auditory behaviors of children. The LEAQ has been in use for several years, which implies that clinicians have placed their trust in its results.

The results showed that questions 1– 4, 8 and 14 had a low correlation with age and a low discriminatory power, these questions were judged to be valuable to the LEAQ as a whole and were therefore kept.

Questions 1–4 are as follows: (1) ''Does your child respond to a familiar voice?'', (2) ''Does your child listen to somebody speaking?'', (3) ''When somebody is speaking, does your child turn his/her head towards the speaker?'', and (4) ''Is your child interested in toys producing sounds or music?''. These 4 items include auditory behaviors that are present in the first 1–2 months of typical child development. This low level of difficulty for items 1– 4 is confirmed in all languages studied, as they are almost always answered with a ''yes'' response.

Questions 8 and 14 are (8) ''Does your child stop crying when you speak to him/her without him/her seeing you?'' and (14) ''When your child is sad or moody, can he/she be calmed down or influenced by music?'' The findings of low correlation with age and low discriminatory power for these two questions may mean that the behavioral responses being assessed by these are more dependent on the individual child (e.g., habits or personality) than on age or auditory skills. More specifically, questions 8 and 14 may not relate to or reflect typical behavior in families. Instead of trying to calm a child auditorily only (i.e., through voice or music alone), parents would be more likely to come close to the child and touch the child while giving auditory support. These visual and tactile components of communication would then be likely to contribute to making the child calm. Thus, the ''auditory-only'' condition which questions 8 and 14 ask about is probably not realistic for most situations or families.

Value could be influenced by providing more examples (question 14) or rewording these questions.

Our study yielded very good results on psychometric measures for the language-adapted versions of the questionnaires. These showed high internal consistency, measuring accuracy, predictive accuracy, and no differences for gender – although has been a fair amount of research documenting a higher (within-family) incidence of language disorders in male children than in female children (Tomblin, 1989; Bishop, 1995; Tallal, 2001;Viding, 2004). The age group the LEAQ assesses may simply be too young for such differences to have fully emerged or the sample size may not be large enough to provide insight into this question. There is some evidence that these genetic (and environmental) differences in language development may be more evident in late toddlerhood (around or after 2 years of age) (Reznick, 1997), which our results of gender independency would tend to support.

Comparing the results of the German version versus the overall results of all the languages (Table XIV), it is apparent that some of the numbers differ quite a bit. This was not particularly concerning because the other groups involved were probably not as highly selected, meaning they were quite possibly more diverse, than the German group. Given the probable homogeneity of the German group, we can accept the scores of the overall group since these more closely resemble real life populations.

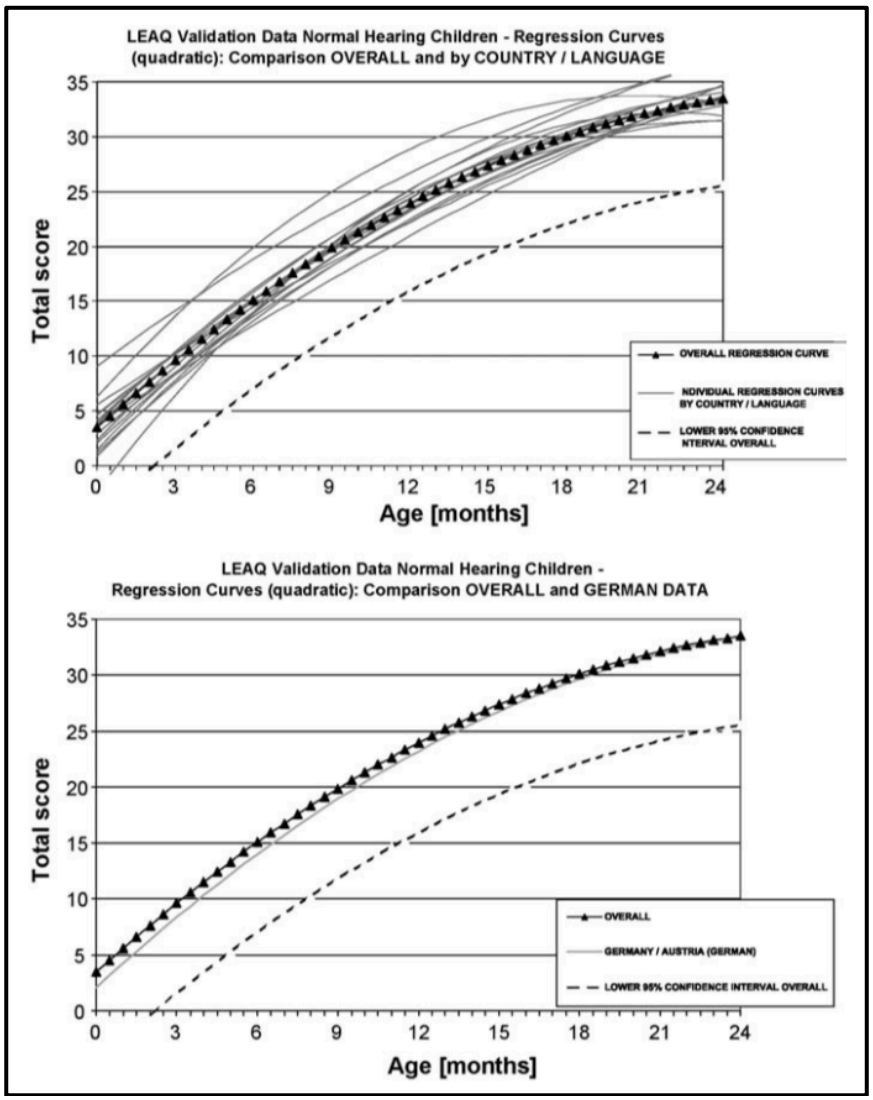

Fig.35 Regression curves (quadratic) with age as independent and total score as dependent variables. (a) Standardized expected values (norm curves) of age- specific auditory behavior for each language and the overall sample. (b) Standardized expected values (norm curves) of age-specific auditory behavior for the German and Austrian versus the overall sample.

Table XIII Standardized expected and minimum values for German

Standardized expected values and standardized minimum values (lower 95% confidence interval) of age-specific auditory behaviour for the German and Austrian versus the overall sample.

Age (months)	Expected value	Minimum value (lower limit of 95% confidence interval)	Age (months)	Expected value	Minimum value (lower limit of 95% confidence interval)
German and Austrian sample					
0 to <1	3	0	12 to <13	24	17
1 to <2	5	0	13 to <14	25	19
2 to <3	7	1	14 to <15	26	20
3 to <4	9	3	15 to <16	27	21
4 to <5	11	5	16 to <17	28	22
5 to <6	13	7	17 to <18	29	23
6 to <7	15	8	18 to <19	30	24
7 to <8	17	10	19 to <20	31	24
8 to <9	18	12	20 to <21	32	25
9 to <10	20	13	21 to <22	32	26
10 to <11	21	15	22 to <23	33	26
11 to <12	23	16	23 to <24	33	27
Overall sample					
0 to <1	5	0	12 to <13	25	17
1 to <2	7	0	13 to <14	26	18
2 to <3	9	1	14 to <15	27	19
3 to <4	11	3	15 to <16	28	20
4 to <5	12	4	16 to <17	29	21
5 to <6	14	6	17 to <18	30	22
6 to <7	16	8	18 to <19	31	23
7 to <8	18	10	19 to <20	31	23
8 to <9	19	11	20 to <21	32	24
9 to <10	21	13	21 to <22	32	25
10 to <11	22	14	22 to <23	33	25
11 to <12	23	15	23 to <24	33	25

Table XIV Overall results of all the languages

Standardized expected mean value (with speech production development according to age) and standardized minimum values (lower 95% confidence interval) of age-dependent speech-production ability. The completed month defines the previous age category.

Age (months)	Expected value	Minimum value	Age (months)	Expected value	Minimum value
0–1	8	5	>9–10	20	14
>1–2	11	6	>10–11	21	15
>2–3	15	7	>11–12	22	16
>3–4	15	8	>12–13	23	16
>4–5	16	9	>13–14	23	17
>5–6	17	10	>14–15	23	18
>6–7	18	11	>15–16	25	18
>7–8	20	12	>16–17	25	19
>8–9	21	13	>17–18	27	19

Furthermore, question 14 is one of only a few questions on the LEAQ which does not include (next to it) an example of the behavior being asked about. Perhaps some examples like ‘‘child stops crying and listens’’ or ‘‘child cries more quietly’’ may be helpful to parents in answering this question. It was decided, though, to keep question 14 because it queries an important topic: music. Even though reactions to music often depend on experience and personal tastes, it was determined that music is an aspect of everyday living that should be polled.

If nothing else, a ‘‘no’’ response to question 14 could encourage communication between the clinician and the caregiver about home practice with music. Though we decided to keep questions 8 and 14, further data would be needed in order to determine their value or if their

One way to improve this study would be to have an equal number of children from each language as well as an equal number of children at each age interval within the samples. As it is now, we see that the language curves (Fig. 35) are very similar, but there are a few curves which stand out slightly. The differences between these curves are not statistically significant.

Conclusions

The results establish the LEAQ as being accurate, gender independent, and age dependent, and also language independent.

The LEAQ is a statistically validated tool currently available for quick and easy assessment of auditory development in children with normal hearing.

Future research should focus on validating the LEAQ in a group of children using hearing instruments. Once validated for this group, the LEAQ could be more widely established as a valid tool for use with infants and toddlers with normal hearing who need follow-up after newborn screening (Coninx, 2008) or with those who have already received very early amplification or implantation.

I. 5. COCHLEAR IMPLANT RELIABILITY

Background

Over the past decade, the adoption of universal hearing screening in newborns has led to earlier detection of hearing problems and significant lowering of the age of first cochlear implantation. As a consequence, recipients are now expected to keep their cochlear implants (CIs) for a longer period of time. Comprehensive longitudinal information on CI reliability is essential for device choice.

Cochlear implants (CIs) restore functional hearing in individuals with profound to severe sensorineural hearing loss. They consist of two main components: the external part (microphone, speech processor, transmitter coil, and batteries) and the implanted part (receiver electronics, magnet and electrode array). The internal components require surgical implantation: the electrode array is inserted into the cochlea and the receiver package is either fixed onto or partly embedded in the temporal bone. Cochlear implantees use their device for daily communication. Most wear their systems throughout the day. Obviously, implanted parts of the CI need to be reliable and remain functional for many years (ideally for the life-time of the patient). In recent years, partly due to increasing hearing screening in newborns, implantation of very young children has become more common (Leigh et al., 2011). This practice has effectively increased the expected lifespan of CIs.

Providing a safe implant on a long-term basis requires that (1) devices must be designed with biocompatible materials, (2) the sterilization process must be effective, (3) design and materials must minimize chronic mechanical tissue trauma and resist mechanical impact, and (4) levels of electrical charge produced by the implant should not exceed those that can be safely supported by human tissues (including neural tissue) without damage. The design and the materials of the implant play a key role in meeting the requirements mentioned above.

The average survival time of implanted CI components has not yet been established. Commercially available CIs have been in routine clinical use for about 20 years, and so the technology is relatively new. Manufacturers typically provide a 10years warranty. Reliability may be reported in terms of average failure rate (FR, i.e., failed to implanted devices ratio). To evaluate the incidence of device failures over time, CI reliability is usually reported in terms of ‘‘cumulative survival rate’’ (CSR), i.e., the proportion of devices still functioning normally after a given time period. This measure is commonly used for other implantable devices such as cardiac pacemakers (ISO Standard, 2000). For CIs, the longest time-period CSR was reported after 12 and 20 years, depending on the manufacturer (Battmer et al., 2009). Survival rates after longer periods are not yet known.

Device reliability is a very important consideration for both surgeons and patients (their parents, to be more specific, as we are talking about children) when considering a particular device. There are currently four main CI systems on the market and it can be hard to get comparable and unbiased CSRs for comparison. Also, CI reliability is a major metric for the manufacturer that can be used to improve their products and manufacturing processes.

Device failure is defined as when the device is not functioning inside the manufacturer’s specification and/or there is no or just insufficient clinical benefit for the patient.1 There also are situations when the device needs to be removed for medical/surgical reasons, such as infection or flap necrosis.

Device failure is classified according to the guidelines of the 2005 Cochlear Implant Soft Failures Consensus Development Conference Statement into hard and soft failures (ISO_Standard, 2000). According to these guidelines, a hard failure refers to detectable

hardware problem and a soft failure refers to underperformance, hearing and/or non- hearing related problems and side effects, or discontinuous function of the device.

One should always keep in mind that the manufacturer's examination of the explanted device takes place after the surgical removal that can, by itself, create a trauma to the device and can result in failure that is sometimes difficult to be distinguished from a previous (pre-explantation) problem. Bearing this in mind is sometimes difficult to accurately defining failures into hard or soft. The reliability of cochlear implants over time is an important issue for doctors and the calculation of cumulative survival rate (CSR) is an objective tool when reporting about this issue. Failure Rate (FR, i.e., failed to implanted devices ratio) is another method to evaluate reliability.

In both studies we followed the ISO reporting standards (ISO 5841-2, 2002) for cardiac pacemakers and we considered the Cumulative Survival Rate a reliable measure that indicates that a device will probably still be functioning after a certain period of time. The Cumulative Survival Rate (CSR) is the cumulative percentage of functioning implant over time and can be used to predict the reliability of the device within a given time period. More specific the FR and the CSR of the Med-El and Digisonic devices were calculated in accordance with the new consensus statement proposed by International Consensus Group for CI Reliability Reporting (i.e., re-implantation following loss of performance - soft failure - if resulted in clinical benefit for the patient was considered device failure).

Several studies have reported reliability in terms of FR and CSR for various CI devices provided by Cochlear Ltd (Sydney, Australia), Clarion-Advanced Bionics LLC (Valencia, CA, USA) and Med-El (Innsbruck, Austria) (Maurer et al., 2005; Battmer et al., 2007; Venail et al., 2008; Battmer et al., 2009; Brown et al., 2009; Soli and Zheng, 2010;). Only FR on a limited number of mixed generations of MXM-Neurelec CIs (Vallauris, France) has been reported in three studies (Maurer et al., 2005; Battmer et al., 2007; Venail et al., 2008) that were not specifically looking at Neurelec data. Over the past 5 years, more and more centers are using Neurelec CIs, thus more comprehensive data regarding the reliability of this system are urgently needed.

All implanted children are expected to wear their devices the entire life – much longer than the 10years warranty offered by manufacturers – so the chance of a device failure or a complication followed by surgical replacement of the device could be a situation experienced by more and more patients.

Personal contribution

Scientific and professional achievements:

Regarding the reliability of the cochlear implants, we have made two multicentric studies: one international related to the CI produced by Neurelec Company and one national regarding the CI produced by Medel. The reliability of cochlear implants over time is an important issue for doctors and the calculation of cumulative survival rate is an objective tool when reporting about this issue. Failure Rate is another method to evaluate reliability. We published the international multicentric study in a ISI journal. Our paper was awarded by UEFISC.

Published papers:

Rădulescu L, Cozma S, Niemczyk C et al.
Multicenter evaluation of Neurelec Digisonic (R) SP cochlear implant reliability. European archives of oto-rhino-laryngology. 2013, (270) 4: 1507-1512. IF = 1,608

Stefanescu EH, Poenaru M, Balica NC, Tudor A, Marinescu A, Georgescu M, **Rădulescu L**, Cozma S, Necula V, Cosgarea M.
Reliability of Med-El Cochlear Implants in children. The Romania Experience. International Journal of Engineering Research and Applications. 2016; 6(7):25-30.

Aim of the study

The aims of the studies were:

- to assess CSR and average FR of the Digisonic SP, the latest generation of CIs released by Neurelec in March 2006, in a large group of implanted adults and children over a time frame of 5 years – implanted in different clinics in the world.
- to assess the reliability of Med-El devices in children throughout Romania as this manufacturer holds a very important share on the Romanian market and worldwide also.

Materials and methods

I present two multicenter retrospective studies that evaluate the reliability of two different cochlear implants devices: Neurelec and MedEL.

Study 1

- a multicenter retrospective case series was conducted in this study, independent of the manufacturer – comprising CI Centers from: Algeria – 1 center, France – 5 centers, Poland – 1 Center, Romania – 1 Center. A questionnaire recording details of implantations was sent (by the first author) to nine CI centers (five in France, one in Poland, one in Greece, one in Algeria, and one in Romania) chosen for using significant numbers of the Digisonic devices in children and adults.

and

Study 2

- a multicenter retrospective study that included 4 major CI centers from Romania requesting information about patients implanted with Med-El devices. Failure Rate (FR) and Cumulative Survival Rate (CSR) over a 5 year period were calculated for this group.
- We did not include the children that have received the cochlear implant in a center outside Romania in this study. The devices included in this study were: Combi 40+, Pulsar and Sonata. All explanted children were re-implanted using Med-El devices. Data regarding the patients requiring re- implantation are shown in Table XV.

Cochlear implantation in all centers was performed using the classic technique with mastoidectomy and posterior tympanotomy. The insertion of the electrode was performed either through a cochleostomy or through the round window. In all cases it was used a double flap technique with either a large or a small incision. The fixation of the device with intraosseous sutures was performed in all but 32 cases when we discuss about MedEl devices and with 2 screws for Digisonic.

The policy of re-implantation consists of using the ipsilateral side whenever possible and to preserve the opposite ear. In case of flap necrosis that could not be reconstructed or

active infection that was nonresponsive to treatment, the device was removed leaving the electrode in the cochlea to prevent cochlear obstruction. The same ear was re-implanted later on. If not possible, the opposite side was considered.

We also evaluated hearing and speech after re-implantation. The speech perception battery included parental LittlEARS questionnaire. We just wanted to evaluate if the initial progress of the re-implanted children continues, but the results of these tests are beyond the purpose of the study.

Inform consent for using patients data in clinical studies was obtained from all patients (parents, care-givers) entering the studies.

We designed a questionnaire to assess the incidence, the time elapsed and the mode of device failure and we sent it to the other three major cochlear implant centers in Romania. We also collected information on reasons for re- implantation and data on explanted devices (serial numbers and manufacturer's technical report) (Table XVI).

The questionnaire recorded the following patient details:

- Date of birth
- Dates of first implantation, explantation, and re-implantation (if applicable)
- Reasons for explantation (medical or apparent device problem)
- Cases lost to follow-up (with reasons and date of the last visit)
- Data on explanted device, including serial numbers, date of manufacture, and technical report from manufacturer documenting test outcomes.

Moreover, in case of re-implantation, centers were asked if outcomes were worse, equal, or better with the new device.

The general method to measure reliability (device failure, survival time, specifications, and classification categories included in the device failure reports) was in accordance with the International Consensus Group for CI Reliability Reporting (Battmer, 2010). Consequently, for explantation following auditory symptoms, non-auditory symptoms, or loss of performance (i.e. ''soft failure'' cases (Balkany, 2005; Balkany, 2005), if clinical benefit was observed after re-implantation, the device was considered failed regardless of the conclusion of technical analysis by the manufacturer.

Consistent with reliability reporting, loss to follow-up was reported in a specific category.

FR is the ratio between failed devices and total implanted devices. CSR was calculated in accordance with the methodology described in ISO standard, 2002. CSR and FR were calculated for all patients, and then separately for the adults and children subgroups.

Results

STUDY 1

From the nine participating centers, 672 patients (362 children and 310 adults) were implanted with Digisonic^{Ò} SP between March 2006 and March 2011 and all were included in the present study. Among these 672 patients, 4 (all adults) were lost to follow up and 15 explantations were noted. Explantations were performed due to device failures in six cases and medical reasons in nine cases.

Four adult patients were lost to follow-up (1.3 % of adult group), one of whom had died from a cause unrelated to the implant, and another was diagnosed with Alzheimer disease.

Table XVI shows the causes for device failures according to the manufacturer's technical analysis and the duration of device use before the occurrence of failure. The causes for device failure included three cases of hermeticity failure (1child and 2 adults; hermeticity compromised the external ceramic case), head trauma (1 child), electrode array malfunction

(one child; 3 electrodes not properly connected to internal stimulator), and one of unknown cause (1 adult; progressive drop in performance, clinical benefit observed after reimplantation with a new device). For this last case, technical analysis did not reveal any implant malfunction.

Nine devices were explanted due to medical reasons (1.35 %). These included three cases of infection of the skin flap covering the receiver/stimulator and one case of cholesteatoma in the implanted ear. Three patients with ossified cochleae were explanted because they had no clinical benefit with the CI (all of them refused re- implantation). One patient was explanted because he had been diagnosed with Neurofibromatosis type 2 after implantation, and one patient was explanted following a loss of clinical benefit (no benefit observed after re-implantation with new device from the same manufacturer). In this last case, the technical report from the manufacturer showed that the device was functioning within specifications.

Table XV Failures rates and cumulative survival rates for children, adults and combined

	Failure rates (%)	Cumulative survival rates
Children ($n = 362$)	0.8	98.48
Adults ($n = 306$)*	1	98.57
Total ($n = 668$)*	0.9	98.51

* Less 4 adults that were lost to follow up

The overall FR was 0.89; 0.83 % for children (patients younger than 18 years), and 0.97 % foradults (Table XV.

Table XVI Number of device failure by failure mode and duration of device use (in months) before use is indicated in brackets

	Hermeticity	Trauma	Electrode array	Electronic	Soft failure
Children ($n = 362$)	1 (5)	1 (14)	1 (48)	0	0
Adults ($n = 310$)	2 (8, 27)	0	0	0	1 (17)
Total ($n = 672$)	3	1	1	0	1

The overall CSR over a 5 year period was 98.51 % (Table XV, Fig. 36), with 98.48 % for children (Table XV, Fig. 37) only and 98.57 % for adults only (Table XV, Fig. 38).

STUDY 2

Overall survival rate of MedEl devices was 95.31%. (Fig.39). Failure rate of MedEl devices is represented in figure 40. There were no significant differences between the four centers regarding the CSR at 5 years (Mantel-Cox log-rank test, p=0.541, significant level α=0.05) but the number of cases was significantly different and the total number of cases is quite small. (Fig.41)

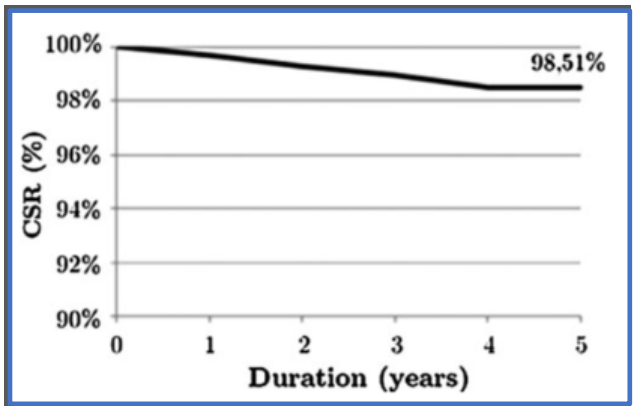

Fig. 36 Cumulative survival rates of all 672 patients over a 5-years period

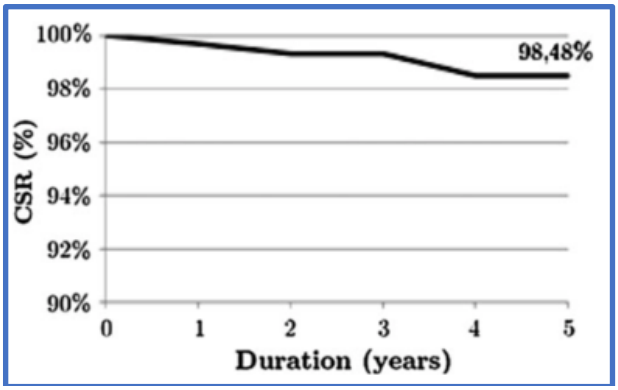

Fig. 37 Cumulative survival rates of the 362 children over a 5-years period

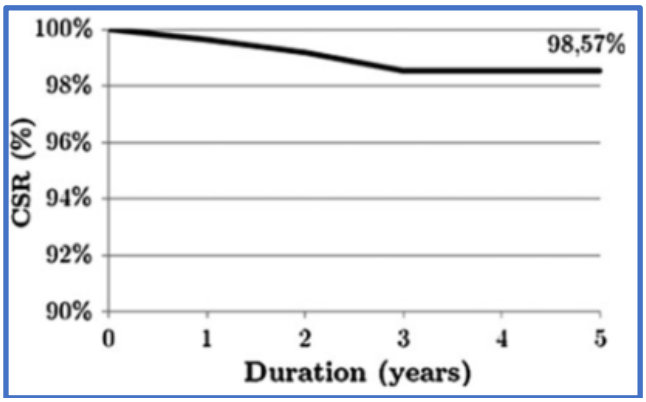

Fig.38 Cumulative survival rates of the 310 adults over a 5-years period

None of the cases was lost to follow-up. There were seventeen (6,64%) cochlear re-implantations in this group with a mean duration of usage before failure of 22 months (range 5–54 months). This was especially the case with Pulsar devices. The number of Pulsar devices that failed exceeded by far the other types of Med-El devices.

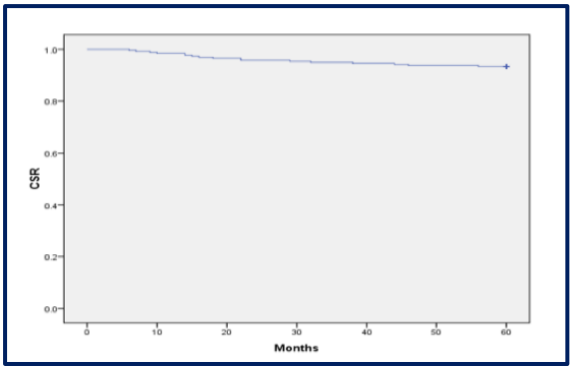

Fig.40 Failure Rate of MedEl Devices

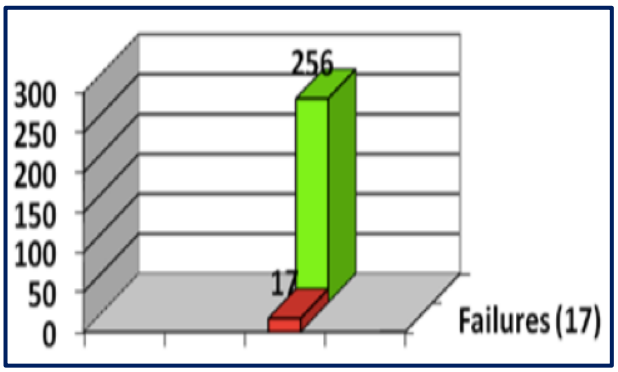

Fig.39 Cumulative Survival Rate at 5 Years. Failed-red; Implanted devices-green

There were 12 device failures and another 5 cases that required re-implantation due to medical/surgical reasons so, in all, 17 devices had to be replaced by the end of the 60 month follow- up period. (Fig.42)

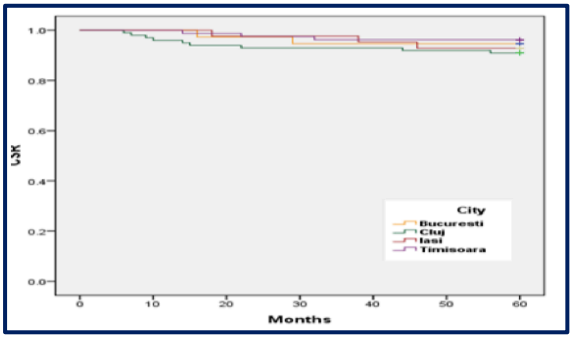

Fig. 41 Cumulative Survival Rates Bucharest; Green-Cluj; Red-Iasi; Purple-Timisoara

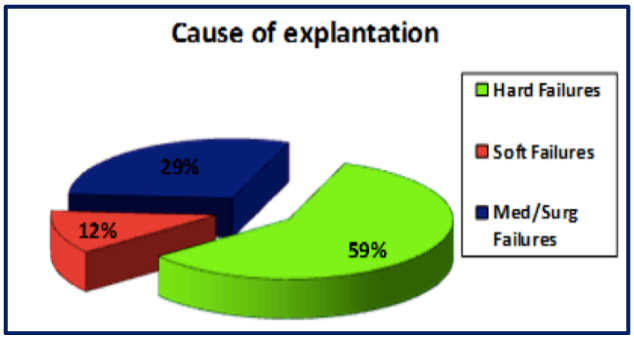

Fig.42 Cause of explantation. Hard Failures-green; Medical/Surgical Failures - blue Flap - related problems Soft Failur.

Hard failures, soft failures, medical/surgical failures- blue, flap-related problems were the main medical/surgical reason for re-implantation.

There was only one case of posttraumatic device failure. We did not find any correlation between meningitis and device failure as none of the children requiring revision surgery had meningitis as cause of deafness.

The type of failure for seven of the devices in the present study was described by the manufacturer as a hermiticity failure. One implant failed secondary to a problem to the ground electrode. In four patients, no clear reason for failure has been found. Four patients requiring re- implantation received a different device model (23%); Regarding functional results –speech perception - all patients performed well after re- implantation continuing, more or less, their initial improvement.

Discussions

Device reliability

Most explantation procedures are carried out following device failure, even though manufacturer analysis does not always confirm a device out of specification (Chung, 2010). Most device failures are spontaneous or due to head trauma (Battmer, 2009), but a small number

(particularly ‘‘soft’’ failures) involved failure breakage of electrode or receiver coil wires due to device/ electrode movement. Besides, duration of device use prior to failure did not show any specific pattern, as failures occurred 5–48 months after implantation. The failure modes observed in the present study appear to be consistent with other reports, though numbers were too small to obtain definitive rates for individual failure modes. Overall, device failure rates from previous studies are mostly in the 3–6 % range; however, as mentioned above, these reports cover a wide range of durations of CI use. A failure rate below 1 % was observed in the present study in a large cohort of Digisonic SP recipients at up to 5 years of device use.

In a previous study on CI failure on a very large number of implanted Cis (Battmer, 2007), mixing all CI generations for each manufacturer (including old generations of CIs) on a long time-span (since beginning of CI programs), the FR for mixed generation of Neurelec devices was 3.2 % (17 cases out of 527), compared to 2 % for Cochlear CIs (617 out of 8,581), 7 % for Advanced Bionics CIs (123 out of 1,761), and 9 % for MedEl CIs (179 out of 1,987). In the present study, the overall FR for Digisonic SP was 6 cases out of 627 (0.97 %). This difference underlines that Neurelec CIs are still improving in terms of reliability.

In this cohort, a CSR of 98.51 % at 5 years was noted. This compares favorably with independently published reports on devices from other manufacturers. For example, it was reported a 5-year CSR of 90.2 % for the Advanced Bionics HiRes 90 K device (Battmer et al., 2009), and 99.6 and 97 % for the Cochlear CI24 device (Battmer et al., 2009; Venail et al., 2008). Such statistics are helpful for CI candidates considering which device to elect for. In this context, it is important that valid comparisons can be made, which can only be ensured by standardized reporting in accordance with the definitions provided by the International Consensus Group (Battmer et al., 2010).

The causes of device failures are of major interest, not least for the manufacturer to implement corrective measures if indicated. In the present study, three device failures were due to hermeticity breakdown. As with other ceramic encased devices (Battmer,2009), the most important rate of the device failures for the Digisonic SP were due to the hermeticity leakage (3 cases out of 6). For MedEL devices, hermiticity problems seemed to be much more frequent and were encountered mostly in ceramic devices. While this failure mode still shows a low rate of incidence, similar problems have been reported for devices from all the other major manufacturers using non-ceramic technology (Brown et al., 2009; Kane and Mann, 2007): for example, Brown et al. (Brown et al., 2009) reported that 31 % (9 out of 29) of device failure was due to hermeticity issues on a group of 806 patients with various CIs. One failure due to head trauma in a child was reported, but numbers were too low to indicate any differences between CSR in children and in adults.

The CSR of the last generation of implants from Neurelec cannot be compared to that of previous generations’ because published data are not available.

Explantation for medical reasons

Many studies have reported on a wide range of complications after CI surgery. Reports distinguished between ‘‘minor’’ and ‘‘major’’ complications, the latter being usually related to issues that require explantation. Minor complications include problems that can be solved with revision surgery without explantation, or without surgery. For example, tinnitus, facial stimulation, and pain can sometimes be relieved by electrode deactivation (Venail, 2008). In the present study, only major complications were reported.

For the first study, revision surgery rates reported in the literature vary from 2.9 % (McJunkin and Jeyakumar, 2010) to 11.2 % (Venail et al., 2008), several factors might account for this range. One revision surgery rate reported specifically for Digisonic SP CI users was 2.4 % (Guevara et al., 2010). A source of variability is the duration of follow-up for studied cohort.

Longer duration will inevitably result in a higher total number of complications, in particular device failure. Even some recent reports include subjects who were implanted before 1990 (Cote et al., 2007), it is possible that prevalence of some failure modes may have changed over time.

Medical complications resulted in nine explantations in this study (1.35 %), the most common of which was 'flap problems. Flap-related problems are reported to be the most common major complication after device failure and appeared to occur in about 1 % of cases in a previous study (Cullen et al., 2008). Strategies are proposed to avoid explantation in infected patients, considering primary immunodeficiency (Yu et al., 2001), but the most important factor appears to be the degree of vascular disruption caused by the surgery. This is clearly evidenced by all studies that have assessed minimal access surgery, for which less flap complications were reported (Ray et al., 2004; Cullen et al., 2008). The present study also included three cases with significant pre-operative ossification, which were eventually explanted due to limited clinical benefit. Strictly speaking, these do not represent either device failures or medical complications, and if these were excluded from statistics then the incidence of explantation due to medical complications would drop from 1.35 to 0.9 %.

Related to the second study:

Re-implantations were performed due to medical/surgical reasons in 5 cases: 3 cases of infection/ necrosis of the skin flap and 2 cases of chronic infection/ cholesteatoma in the implanted ear. Flap-related problems are reported to be the most common complication after cochlear implantation. The cause of flap related problems seems to be the degree of vascular disruption caused by the surgery. This is supported by all studies that have assessed minimal invasive surgery, for which less flap complications were reported (Ray, 2004; Cullen, 2008). In our study more than half of the devices had ceramic housing and a large incision was used (except for the 32 cases already discussed and for the non-ceramic ones).

Revision surgery implies at least the same risks and complications as the first operation. As we do not know how many times we need/can successfully replace the electrode array, the insertion of the electrode should be as gentle as possible and every surgery as atraumatic as possible. The caliber of the intra cochlear tract is strongly dependent on the diameter of the explanted array, and so we re-implanted with arrays of the same diameter whenever possible (76% of cases).

In this second study none of the patients requiring CI re-implantation had bacterial meningitis as the cause of hearing loss. Overall meningitis as an etiologic factor accounts for 6% of all patients in our group. The failure rate in children with meningitis in our study was 0%, which is not common. According to other reports, this usually exceeds the failure rate in children in whom deafness resulted from other causes.

Reliability reporting

There are multiple factors reflected in the CSR number. These include patient characteristics (age, malformations of the cochlea, ossification of the inner ear, etc.), features of the implanted device, surgical technique, and surgical skill. Taking these individuals and often interconnected factors apart is not possible.

The same standards and factors have to be followed by all studies reporting device reliability, thus making the results comparable to guide patients, clinicians, CI centers, and manufacturers. To be truly useful, a study regarding CI reliability would require:

– Conformance with International Consensus Group guidelines (Balkany et al., 2005; Battmer et al., 2010).

– The reliability measure has to be quantified with common methods—CSR and FR in the present study.

– Each and every CI model has to be assessed separately to compare reliability between them.
– The duration of device use must be long enough to be consistent in assessing the device FR. Counter-intuitively, a previous study has shown that 24 % of re-implantations due to device failure or infections occurred before 2.5 years and 72 % of them before the fifth year of implantation, suggesting that the longer the follow-up time, the smaller the number of re-implantations (Venail et al., 2008).
– Device failure data has to be systematically reported by all CI centers.
– Device failure assessment has to be conducted on a large number of implants. As observed by Battmer et al. (Battmer et al., 2009) the size of the studied cohort varies extremely from one study to another, influencing statistical robustness—smaller samples creating the greater uncertainties. In addition, multicenter studies should be employed when a specific model of device is assessed to avoid any peculiarities linked to an individual center.
– The centers participating in studies must be chosen randomly. Cochlear implantation is performed by otologists with widely ranging skill levels and the study must give a reasonable impression regarding device reliability even in the hands of less experienced surgeons. The relation between FR and surgical skills was already emphasized in medical literature (Cohen, 1995). The inclusion of centers with surgeons showing different levels of experience in cochlear implantation strengthens the results of reliability studies.

Revision surgery implies at least the same risks and complications as the first operation. As we do not know how many times we need/can successfully replace the electrode array, the insertion of the electrode should be as gentle as possible and every surgery as atraumatic as possible (Chung et al., 2010). The caliber of the intra cochlear tract is strongly dependent on the diameter of the explanted array, and so we re-implanted with arrays of the same diameter whenever possible.

Conclusions

The FR was better when compared to previous generation of Neurelec CIs and was comparable to FR from other manufacturers. CSR was found to be comparable or better, in specific cases, to that of other CIs available on the market.

In order to allow comparison between different CI systems' reliability studies, it is crucial to keep the rules for device failures reporting and for CSR calculation unchanged in the future.

Also, one should always keep in mind that the manufacturer's examination of the explanted device takes place after the surgical removal that can, by itself, create a trauma to the device and can result in failure that is sometimes difficult to be distinguished from a previous (pre-explantation) problem. Bearing this in mind is sometimes is difficult to accurately define failures into hard or soft.

II. FUTURE PROJECTS

A GLIMPSE INTO THE FUTURE TREATMENTS OF THE HEARING LOSS

II.1. PERSONALIZED MANAGEMENT OF DEAF CHILDREN

My goal is to continue the work related to the management of hearing loss.

Background

Since its introduction, the cochlear implant has been continuously improved in terms of stimulation and coding strategies, indications of implantation, surgical technique and design of the device itself.

Improvements in stimulus and coding strategies are related to the construction of electrodes. The electrodes may be straight - they are more flexible but due to the greater distance between the columella and the electrode the current consumption is higher, which means a higher battery usage and a possible interaction between the electrodes.

Another type of electrode is curved electrode, which is capable to embrace the columella, consuming less energy with a better stimulus result, avoiding the interaction between the channels.

With regard to the coding strategy currently, most implants use the CIS coding strategy.

Improvements in the implant itself have been made by miniaturization, and by choosing the best possible materials from which it is made.

One of the most important changes in implant indications was the decreasing the implantation age under 1 year. Theoretically, there is no lower limit because the earlier the implant is, the better the results - the children implanted under the age of one year have the same level of language development as a normal hearing child.

The introduction of bilateral implantation represented another improvement in the auditory capacity of cochlear implant recipients by allowing sound source orientation and improving audition in noise environments.

Improvements have been made also in the surgical technique the incisions are smaller, insertion of the electrode has been improved by lubricating it with hyaluronic acid or with Dexamethasone, the slow insertion of the electrode avoids damage to the structures in the cochlea.

The main limitation of the cochlear implant refers to the difficulty of speech understanding in noise.

The main unanswered question is the high variability of the outcomes in apparently homogenous groups of implanted children.

At this time, however, the cochlear implant by electrical stimulation of the auditory nerve remains the only viable solution for profound loss rehabilitation.

The main objective is to improve the outcome of cochlear implanted children, to decrease the rate of the complications and to give them a better chance to be part of the " hearing society".

I am working in a complex research team well trained and with good knowledge in hearing loss pathology.

- I will continue the research that was initiated in the OTIC-gene research project. To identify the factors that are responsible of the outcome of the implanted congenital deaf children.

- I am interested in evaluating the outcome of simultaneous implanted vs sequential implanted children and also to establish the optimum interval between the two surgical cochlear implantations in cases of sequential.

- I am also concerned with evaluation of biofilm formation on cochlear implants and to find modalities to decrease it:
  - For the beginning I intend to functionalize the silica cochlear implants with N-Acetylcysteine – followed by assessment - in vitro – of the biofilm adherence to the new created surface compared to the silica cochlear implant. The final objective is to improve the properties of the implant such that the rate of biofilm formation to become lower;
  - I intend to continue with the characterization of the microbiota of the healthy middle ear;
  - Finally, I am interested to study the mechanism of biofilm formation on the cochlear implants in an experimental study.

Bacterial biofilm formation is considered to play a major role in the pathogenesis of biomaterial associated infections (Costerton et al., 1999; Holmberg et al., 2009).

The use of medical devices (catheters, artificial heart valves, prosthetic joints, cochlear implants etc.), increased dramatically over the past century (Rabih and Darouiche, 2004) and has become a major part of modern medicine and our daily life. The risk for BAI may in part be explained by the reduced efficacy of the local immune defense induced by the foreign body. It was shown that the number of bacteria required to cause an infection is significantly lower in the presence of a foreign body, such as in case of implants than when such devices are not present.

Cochlear implants are considered nowadays standard of care for patients (children and adults) with profound sensorineural hearing loss.

There were an estimated 34–36 million adults with measurable hearing loss in the United States in 2009 (Kochkin, 2009).

Of that number, 1.2 million children and adults (with severe to profound hearing loss) were thought to be potential implant candidates (iData, 2010). The total number of CI recipients in the United States in 2009 was estimated at 70 000 adults and children (iData, 2010) and around 170.000 world-wide.

Development of infectious complications after cochlear implantation is rare, with an incidence of 1% to 4% (Johnson et al., 2007). The precise mechanism of infection is unknown.

II.2. TREATMENT OF HEARING LOSS WITH NANOPARTICLES

Background

From literature survey, it is evident that the intra-tympanic route remains the most appropriate route to deliver drugs in the inner ear.

In order to increase the effectiveness of the treatment, several types of drug delivery systems were studied. The first challenge is to ensure the safety of the drug carrier to the middle inner ear. Starting from this constraint, carriers based on biocompatible inorganic materials (silica), organic materials (liposomes, lipids, dendrimers), biodegradable natural-based polymers (gelatin, hyaluronic acid, alginate, chitosan, etc.) and synthetic polymers were prepared.

Applications for inner ear drug delivery are focused on four main fields: otoptotection, hearing loss, autoimmune inner ear disease and regeneration of hair cells.

This project proposal aims is to develop new drug-loaded nanocarriers designed to treat some inner ear disorders, capable of active targeting and internalizing in its specific cells. Two types of nanocarriers will be studied within this project: chitosan-based nanocapsules and liposomes.

The advantage of these nanocapsules, recently obtained by interfacial condensation between chemically modified chitosan and an alternating copolymer of N-vinyl pyrrolidone with itaconic anhydride [poly (NVPAI)] by the team from the Apollonia University, is based on higher drug encapsulation efficiency as compared to nanoparticles of Hydrogels type (nanospheres). The advantage of liposomes consists in the fact that both hydrophilic and hydrophobic drugs can be loaded in this type of nanocarriers.

In order to achieve efficient active targeting of internal hair cells, spiral ganglion cells, supporting cells in the cochlea, and ensuring a high efficacy of the treatment, our approach proposes a combined method consisting in the realization of hybrid nanocarriers loaded with superparamagnetic nanoparticles (maghemite) but also functionalized in the surface with ligands readily recognizable by cell-specific receptors in the inner ear.

The magnetic behavior of these two nanocarriers (nanocapsules and liposomes) will allow their active targeting and thus their concentration in the internal ear, under the action of an magnetic external field. After the drug release and the biodegradation of the nanocarriers, the maghemite NPs might be extracted from the body by using an outer magnetic field.

The functionalization of the nanocarriers with specific ligands will enable their internalization in the cells from the inner ear. Short-chain peptides will be used as ligands, their selection being based on the chemical bonding ability of the nanocarriers membrane. Ligand-functionalized nanocapsules will be obtained using a mixture of non-functionalized and functionalized oligochitosan. The functionalized oligochitosan will be prepared by a amidation reaction between the COOH groups of the ligand and the NH_2 groups of the oligochitosan.

Ligand-functionalized liposomes will be prepared after the preparation of the liposomes by a reaction between the SH groups of the ligand and the maleimide groups of the phospholipides.

Obtaining these hybrid (magnetic) nanocarriers will be achieved by encapsulating the maghemite NPs during the preparation phase. The maghemite NPs will be dispersed in the aqueous phase with which the hydration of the lipid film is performed, respectively, in the slightly acidic aqueous solution of chitosan which will be subsequently interfacially condensed with the alternating poly (NVPAI) copolymer.

The drug loading will be accomplished in the nanocarriers preparation step by dissolving/dispersing the drug in the aqueous medium. Two drugs will be studied, such as an

anti-inflammatory and an antimicrobial, as the primary ear disorders that lead to hearing loss are inflammation and infection.

The obtained hybrid nanocarriers will be structurally characterized (spectral methods), physicochemical, morphological (electron microscopy), from the point of view of the biomaterial properties (cytotoxicity, hemocompatibility, etc.), and the loading capacity and especially the drug release, biodegradability under simulated internal ear conditions (for example, chitosanase for polymeric nanocapsules) will be also studied.

From the literature survey it appeared that the most suitable administration route of the drug-loaded nanocarriers is the intra-tympanic injection therefore in this project this route will be used.

The biodistribution of the administered nanocarriers to the internal ear will be analyzed after their prior functionalization with markers (rhodamine, fluorescein) by confocal fluorescence microscopy after sacrificing the experimental animal (chinchilla, selected for similarity to its internal ear with the human ear).

Evidence of in vivo biodistribution of the nanocarriers will be achieved by NMR. The participants in the consortium were selected on the principle of their complementarities, each of them having specific tasks to solve within the project.

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