ABSTRACT PhD THESIS

SOURCES OF PREANALYTICAL ERRORS - NEGATIVE IMPLICATIONS IN SCREENING OF HEMOSTASIS AND MONITORING OF ANTICOAGULANT THERAPY

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Univ.Prof.PhD. AGOP Maricel
„Alexandru Ioan Cuza” University from Iași

PhD Student:
ȘOLOGON (DELIANU) Carmen

2020
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<td></td>
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</tr>
</tbody>
</table>
NOTE:

The summary of the PhD thesis presents the results of personal experimental research, general conclusions, and a selective bibliography.

The abstract keep the same numbering used in the text of the doctoral thesis for chapters, tables, and figures.

This PhD thesis comprises a number of 82 figures, 34 tables, and 330 bibliographical references. The abstract includes a limited number of figures and tables, maintaining the numbering in the doctoral thesis.

KEYWORDS:

- Hemostasis,
- Coagulation tests,
- Sources of error,
- *In vitro* hemolysis,
- *In vitro* clot,
- Clinicians information,
- Training of nurses.
PERSONAL PART

CHAPTER VII

INTERFERENCES BETWEEN CLOT IN THE ERYTHROCYTE SEDIMENT AND HEMOSTASIS EXPLORATION

Introduction
In treating patients with thrombotic or hemorrhagic disorders and determining a correct diagnosis, laboratories performing hemostasis tests play an important role (Mohammed et al., 2019).

Objectives
The aim of our retrospective study is to draw attention to the abounding situations in which the clot cannot be identified before centrifugation, but instead, its accidental presence in the erythrocyte sediment generates negative interferences with the determination of PT and aPTT coagulation tests.

Material and method
This study was conducted in the Hematology Department of the “Sf. Spiridon” County Emergency Clinical Hospital Iasi, Romania, during a 4-months period. The samples were collected in vacutainer embedded with Na citrate, respecting the ratio of 1-part anticoagulant: 9 parts sampled blood (Harris, 2012, Ellouze, Guermazi, 2013, Magnette et al., 2016, Polack et al., 2001, Mackie, 2013).

The determinations were performed on a ACL TOP 500 automatic analyzer. Reagents from HemosIL Instrumentation Laboratory, RecombiPlastin 2G for PT and APTT-SP (Synthetic Phospholipids) for aPTT were used.

All data has been analyzed with SPSS test v.24 (IBM SPSS Statistics). For the continuous variables, statistical benchmark indicators (mean, 95% confidence interval for mean, standard deviation, min, max, etc.) were presented.

Results
In order to study the impact of the accidental presence of clot on coagulation tests determinations, respecting the inclusion criteria, only the 153 (22.8%) samples, which were post-analytically identified with clot in the erythrocyte sediment, were analyzed. For these samples, we used the procedure of postanalytical re-verification by transfer, described in fig. 6.30.

Fig. 6.30. Highlighting the postanalytical in vitro clot through the transfer procedure (OSIM - personal collection) (Delianu et al., 2017, 2018)
The interpretation of the results was performed by framing the PT and APTT values in the following reference intervals:

Table 7.1. Reference intervals (sec)

<table>
<thead>
<tr>
<th>REZULTATE</th>
<th>PT</th>
<th>aPTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>scuritate</td>
<td>≤ 9.9``</td>
<td>≤ 19.9``</td>
</tr>
<tr>
<td>normale</td>
<td>(10<code> - 14</code>)</td>
<td>(20<code> - 40</code>)</td>
</tr>
<tr>
<td>terapeutice</td>
<td>(14.1<code> - 69.9</code>)</td>
<td>(40.1<code> - 85.9</code>)</td>
</tr>
<tr>
<td>pretungite</td>
<td>≥ 70``</td>
<td>≥ 86``</td>
</tr>
<tr>
<td>„FAILED”</td>
<td>undetectable</td>
<td>undetectable</td>
</tr>
</tbody>
</table>

Table 7.2. Comparison between the frequency of PT₁ / APTT₁ results from the post-analytically identified samples with sediment clot and PT₂ / APTT₂ after re-sampling

<table>
<thead>
<tr>
<th>PT₁</th>
<th>PT₂</th>
<th>Kappa Acord (k)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 9.9``</td>
<td>10<code>-14</code></td>
<td>14.1<code>-69.9</code></td>
<td>≥ 70``</td>
</tr>
<tr>
<td>undetectable</td>
<td>1(0.8%)</td>
<td>9(6.8%)</td>
<td>7(5.3%)</td>
</tr>
<tr>
<td>≤ 9.9``</td>
<td>2(1.5%)</td>
<td>19(14.4%)</td>
<td>-</td>
</tr>
<tr>
<td>10<code>-14</code></td>
<td>23(22.7%)</td>
<td>3(0.8%)</td>
<td>1(0.8%)</td>
</tr>
<tr>
<td>14.1<code>-69.9</code></td>
<td>10(7.6%)</td>
<td>33(25.0%)</td>
<td>-</td>
</tr>
<tr>
<td>≥ 70``</td>
<td>1(0.76%)</td>
<td>10(7.58%)</td>
<td>12(9.09%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>aPTT₁</th>
<th></th>
<th>20<code>-40</code></th>
<th>40.1<code>-85.9</code></th>
<th>≥ 86``</th>
</tr>
</thead>
<tbody>
<tr>
<td>undetectable</td>
<td>23(22.8%)</td>
<td>8(7.9%)</td>
<td>3(3.0%) FN</td>
<td></td>
</tr>
<tr>
<td>≤ 19.9``</td>
<td>25(24.8%)</td>
<td>-</td>
<td>-</td>
<td>0.097</td>
</tr>
<tr>
<td>20<code>-40</code></td>
<td>23(22.8%)</td>
<td>-</td>
<td>-</td>
<td>0.097</td>
</tr>
<tr>
<td>40.1<code>-85.9</code></td>
<td>6(5.9%)</td>
<td>4(4.0%)</td>
<td>-</td>
<td>0.097</td>
</tr>
<tr>
<td>≥ 86``</td>
<td>25(24.75%)</td>
<td>14(13.86%)</td>
<td>14(13.86%)</td>
<td>4(3.96%)</td>
</tr>
</tbody>
</table>

(*) Marked effects are significant at p <0.050;
FN-fals negativ; FP-fals pozitiv;

Figure 7.3. Relationship between the modifications of the (PT₁) test results observed in the clot samples in the erythrocyte sediment, after the repetition of the determinations (PT₂) of new collected samples
Figure 7.4. Relationship between the modifications of the results of the \((aPTT_1)\) tests observed in the clot samples in the erythrocyte sediment, after the repetition of the determinations \((aPTT_2)\) of new collected samples.

Table 7.3. Statistical indicators for PT and aPTT values at the two analyzed stages

<table>
<thead>
<tr>
<th></th>
<th>Means</th>
<th>Means -95%</th>
<th>Means +95%</th>
<th>Std. Dev.</th>
<th>Std.Err.</th>
<th>Min</th>
<th>Max</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT_1</td>
<td>33.9</td>
<td>25.9</td>
<td>41.9</td>
<td>43.0</td>
<td>4.0</td>
<td>8.4</td>
<td>234.4</td>
<td>10.5</td>
<td>14.0</td>
<td>39.8</td>
</tr>
<tr>
<td>PT_2</td>
<td>26.5</td>
<td>20.6</td>
<td>32.3</td>
<td>33.9</td>
<td>2.9</td>
<td>9.7</td>
<td>225.8</td>
<td>11.0</td>
<td>13.1</td>
<td>23.6</td>
</tr>
</tbody>
</table>

*Levene Test of Homogeneity of Variances: \(F=4.84, p=0.0287^*\)

*Kruskal-Wallis-H = 0.0189, p = 0.8907*

<table>
<thead>
<tr>
<th></th>
<th>Means</th>
<th>Means -95%</th>
<th>Means +95%</th>
<th>Std. Dev.</th>
<th>Std.Err.</th>
<th>Min</th>
<th>Max</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT_1</td>
<td>43.1</td>
<td>32.4</td>
<td>53.9</td>
<td>44.1</td>
<td>5.4</td>
<td>14.4</td>
<td>255.5</td>
<td>18.9</td>
<td>25.8</td>
<td>43.0</td>
</tr>
<tr>
<td>aPTT_2</td>
<td>38.2</td>
<td>32.9</td>
<td>43.5</td>
<td>26.9</td>
<td>2.7</td>
<td>20.4</td>
<td>240.9</td>
<td>26.4</td>
<td>29.7</td>
<td>35.1</td>
</tr>
</tbody>
</table>

*Levene Test of Homogeneity of Variances: \(F=11.51, p=0.00086^*\)

*Kruskal-Wallis-H = 7.37, p = 0.0066*.

*Statistical test - 95%CI (Confidence Interval) – (*)Marked effects are significant at \(p < 0.05\)*

Discussions

The goal of oral anticoagulant therapies is to minimize the incidence of hemorrhagic complications and the risk of thromboembolic events, by maintaining the therapeutic intervals of PT, reported in the INR (van Geest-Daalderop et al., 2005).

The sources of error generated by the sampling of biological products can have particularly serious consequences for patients and the healthcare process, since 60 to 80% of medical decisions are based on laboratory tests results (Forsman, 1996, Katayev et al., 2010, Green, 2013, Nikolac et al, 2013).

Nikolac et al. mention that hemostasis testing can be affected by tissue factors or clot activators during sampling, which is why these samples should be collected before introduction in tube with anticoagulant (Nikolac et al, 2013).
In the analyzer, the centrifuged samples are positioned in the pre-analytical area. For testing, the plasma is automatically drawn from the sample and released into the cuvette in the analytical area of the analyzer.

The moment when the automatic pipette of the analyzer aspirates fibrin (fig. 7.7) or air bubbles from the supernatant (fig. 7.8), is blocked by them, the recommended amount of plasma for the determination of 0.1 ml coagulation tests being not properly acquired, generating false results therefore.

**Fig. 7.7.** *The presence of fibrin in the supernatant obstructs the aspiration of the recommended amount of plasma for the determination of coagulation tests*

Chawla and colleagues mention that special attention should be paid to the identification of fibrin in the supernatant, recommending the adoption of sample handling protocols, as its presence can cause blockage of the analyzer suction pipettes, leading to erroneous coagulation test results (Fig. 7.8) (Chawla et al., 2010)

**Fig. 7.8.** *The presence of air bubbles in the supernatant obstructs the aspiration of the recommended amount of plasma for the determination of coagulation tests*

Based on the results of falsely low or prolonged coagulation tests, in the case of patients monitored for anticoagulant therapy, incorrect dosing may occur, and therefore
depending on the direction of error, the patient may be placed at undue risk of bleeding or thrombosis (Favaloro et al., 2012). In the case of routine coagulation testing, the results of false "screening" tests can influence the clinical decision, generating discomfort for the patient, as a result of subsequent investigations (Favaloro et al., 2012).

By total coagulation of the collected blood, in some situations, the prolongation or shortening of the tests is the result of performing determinations in the serum due to the consumption of coagulation factors (Favaloro et al., 2012). As a remark, based on these considerations, the re-verification of the post-analytical samples is also justified to exclude the accidental presence of the clot as is the case of undetectable results either.

Such results are canceled and it is recommended to repeat the sampling and implicitly the determination of the PT and aPTT tests. The statistical significance of the differences obtained between the results at the two moments could be considered an important criterion, regarding the completion of the patient's clinical picture, a proper diagnosis and last but not least, the therapeutic doses based on false results.

In 2017, Duncan and Rodgers drew attention to the fact that the evaluation of the activity of coagulation factors is carried out by measuring the capacity of an unknown sample, being important for laboratory specialists to understand the interpretation and limitation of results and the impact of variations, these aspect worthing to be analyzed (Duncan, Rodgers, 2017).

Kamal and colleagues in their study mentioned that in the event of prolonged results of PT and aPTT tests recommended for preoperative screening or initial assessment of bleeding, a dilemma arises in the diagnostic algorithm, requiring an analysis of key factors that would affect test results, thus providing suggestions on the appropriate tests and recommended situation that claims specialist consultation (Kamal et al., 2007). The clinician is the one who must determine whether the extension of PT and aPTT tests is artificial, or they can be correlated with hemostatic abnormalities or are related to medication (Kamal et al., 2007).

However, we consider that in the case of false positive results, highlighting a coagulation disorder and a false bleeding risk due to the presence of clot in the erythrocyte sediment (Table 7.2), the severity of the consequences would have been unfavorable for the patient's health due to the major differences between the obtained result and the real one, given that these analyzes are often considered "diagnostic".

Our study reveals that there are still unsafe areas in the post-analytical phase that need to be explored. Results that cannot be correlated with the evolution and the clinical picture of the patient can be obtained from apparently normally collected samples. The reason for improving the procedures for postanalytically identifying clots in the sediment of samples is to provide accurate results, of pivotal importance for the patient care.

Conclusions

This transvasation procedure can be used in all cases where the presence of the clot in the erythrocyte sediment is suspected. It is worthwhile for all healthcare professionals to be aware of the negative impact their reporting may have, before becoming critical to the patient.

Achieving new reverification procedures, which would guarantee clinicians the use of laboratory results with maximum confidence, would be a great challenge for each medical laboratory.
CHAPTER VIII

BLOOD CLOT IDENTIFICATION PROCEDURES IN CLINICAL LABORATORY SAMPLING

Introduction
Haemostasis is one of the disciplines of biology that is totally dependent on the pre-analytical phase in the clinic, starting with patient preparation, biological product collection, manipulation and, last but not least, specimens transport (Magnette et al., 2016). This phase is considered to be the most vulnerable part of the testing process (Loeffen et al., 2012, Cornes et al., 2016), as it contains many manual activities, sources of non-interchangeable or inaccurate results which in turn, could be reduced by standardizing the pre-analytical stage (Kitchen et al., 2013, Guder, Narayanan, 2016, van Dongen-Lases et al., 2016).

Objectives
The purpose of this study is to present the procedures used to highlight the accidental presence of the clot. The objectives envisage identification of the recommendations from the literature, based on the preclinical studies in which, both pre-analytical and post-analytical procedures operated.

Materials and methods
We conducted a search on PubMed, Google Scholar and Cochrane to adjust a comprehensive approach to pre-analytical and post-analytical procedures recommended and used in laboratories performing PT and APTT coagulation tests. To illustrate identification procedures, we used word-drawn pictures and pictures that capture platelet aggregates, fibrin and clot when examining a venous blood smear. We explored the most recent studies describing the incidence of clot specimens, the manner it is formed, and the procedures used to identify the clot. We took into consideration study protocols, reported cases, and opinion polls.

Results
During our research, we have been able to find articles and laboratory guides that explain how to prevent clotting in vitro (OPTMQ, 2006, OPTMQ, 2011, Favaloro et al., 2012, Ellouze, Guermazi, 2013, Krleza et al., 2015, Magnette et al., 2016), as well as studies presenting procedures for verifying suspected clot samples (Ernst, 2004-2007, OPTMQ, 2008, Favaloro et al., 2012, Delianu et al., 2017, 2018).

We have also taken into account studies that mention the negative impact of fibrin on the results and implicitly of the analyzers (Chawla et al., 2010). There have been studies reporting the deficiencies in the pre-analytical use of verification procedures were reported (Ernst, 2004-2007).

Discussion
In the national medical literature of the clinical laboratory, in 1981, Kondi considered that partially coagulated blood is one of the technical errors, which could be the cause of a diagnosis of a prolonged PT (Kondi, 1981). Seventeen years later, Enache and Stuparu recommended checking the erythrocyte sediment carefully, to highlight the presence of small clots, in the case of samples taken on an anticoagulant, considered a situation in which the sample should not be processed (Enache, Stuparu, 1998).
In 2006, American pathologists recommended the visual highlighting of the clot (CAP, 2006, OPTMQ, 2008), through the slight inversion of the specimen (fig. 7.1), a procedure used today in most laboratories.


![Fig. 8.1. Wood applicators for clot identification](image1)

American College recommends highlighting the platelet aggregation through the microscope (fig. 8.2), the fibrin (fig. 8.3) or clot (fig. 8.4), by displaying a venous blood smear (CAP, 2006) May-Grunwald-Giemsa colored.

![Figure 8.2. Platelet aggregates on venous blood smear (personal collections)](image2)
Paying special attention to the reliability of the results released to clinicians, starting with 2014, in our laboratory we implemented postanalytic and transfer procedure (PS-H-04, 2014, PS-H-04-01, 2018) (Delianu et al., 2017, 2018), (fig. 6.30) in order to highlight the clot in the sample, whenever the results obtained do not correlate with the evolution of the patient’s results.

On the same principle, Petri dishes (Fig. 8.5) or small plastic funnels fitted with a paper or gauze filter (Fig. 8.6) may be used for "control" instead of test tubes, in order to retain the clot from the transferred blood.
Figure 8.5. Blood transvasation from the primary sample through a plastic funnel (b)

Not to be neglected are the additional costs that involve the purchase of test tubes, Petri boxes and plastic funnels for the upper mentioned procedures. However, performing determinations from partially coagulated samples leads to the release of false results in case of anemia, bicytopenia or pancytopenia depending on the consumption degree of the cell elements, for the determination of the CBC (Favaloro, Lippi, 2008, OPTMQ, 2011, Favaloro et al., 2012). In the case of coagulation determinations from samples containing fibrin or clot, depending on plasma factors consumption, false prolonged time can be recorded.

Conclusions
It remains challenging for each laboratory to be able to settle down its own pre-analytical and post-analytical identification of the clot accidental presence in the sample used for coagulation tests, complete blood count and erythrocyte sedimentation rate (ESR) determinations. In this context, the presence of clot in the sediment of the sample may produce false results of hypercoagulability or hypocoagulability depending on the partial or total consumption of coagulation factors (I, II, V and VIII), leucopenia, anemia, thrombocytopenia depending on the cellular elements consumption, such as leukocytes, erythrocytes or platelets. Results related to a false inflammatory process in the case of a false elevated ESR test can arise as well, as they cannot be correlated with the real clinical condition of the patient.
CHAPTER IX

PROTOTYPE DEVICE USING ULTRASOUND FOR IN VITRO CLOT HIGHLIGHTING

Introduction

Differential methods suffer when "reality" operates with instabilities, instabilities that can generate both chaos and self-organization in the dynamics of the blood. This means that any variable that is classically dependent on both spatial and temporal coordinates becomes, in this context, also dependent on scale resolutions. More precisely, as long as we operate with a variable, described mathematically by a strictly indistinguishable function we operate with different approximations of this function at various scale resolutions.

Objectives

Starting from the Doppler principle and the undisputed applicability of ultrasonography for vascular exploration, we aimed to experiment with the identification of clot in vitro, to add value to Laboratory Medicine, in terms of extending innovative procedures to highlight accidental presence of the clot and in the erythrocyte sediment (in vitro) using ultrasound.

Material and methods

To document the applicability of ultrasound in the blood coagulation process, a literature review was performed using PubMed, Google Scholar, and Cochrane database records. The selection of studies from the period (2019-1997) was abstracted using a table, starting from the design method, objectives, evaluation methods, and conclusions; default based on keywords: ultrasonic, coagulation, clot, plasma, serum, as described in Table 9.1.

Table 9.1. Relevant articles included in the study

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Title</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>Callé R, Plag C, Patat F, Ossant F.</td>
<td>2009</td>
<td>Interest of the attenuation coefficient in multiparametric high frequency ultrasound investigation of whole blood coagulation process.</td>
</tr>
<tr>
<td>Huang CC1, Wang SH.</td>
<td>2007</td>
<td>Assessment of blood coagulation under various flow conditions with ultrasound backscattering.</td>
</tr>
<tr>
<td>Calor-Filho MM, Machado JC.</td>
<td>2006</td>
<td>Measurement of the ultrasonic attenuation coefficient of human blood plasma during clotting in the frequency range of 8 to 22 MHz.</td>
</tr>
<tr>
<td>Machado JC, von Kruger MA, Fontes EM, de Almeida MM.</td>
<td>1997</td>
<td>Evaluation of an ultrasonic method applied to the measurement of blood coagulation time.</td>
</tr>
</tbody>
</table>
Results

The blood was assimilated into a complex fluid whose entities move on continuous and non-differential curves (multifractal curves). As the fundamental property of these curves is self-similarity (the part reflects the whole and vice versa - ie a property specific to all biological structures) we proposed a mathematical model based on this property to serve blood dynamics from in vivo to in vitro.

According to equation (2), the blood flow domain can be separated into two abstract subdomains: subdomain 1, where the speed of shearing blood is high, and the blood tends to a Newtonian behavior, and, respectively, sub domain 2, where the blood flow is dominant. In this subdomain, the blood moves at a constant speed in the form of a solid plug, which in our opinion would correspond to the clot. In this region $r_0 > \eta \frac{\partial U}{\partial r}$.

![Diagram of a Bingham fractal fluid with fractal stress and velocity fields](image)

**Fig. 9.4. Schematic representation of a Bingham fractal fluid. Fractal stress diagram (a) and fractal velocity fields (b)**

The analysis of stationary blood dynamics on a differential scale based on the transition in vivo - in vitro under the action of an external constraint (manifested in our case in the form of blood sampling) involves Bingham-type rheological behaviors of the blood. In
such a context, the virtual germs (fig. 9.5 a, b, c, d) are self-structured by coherence in “vortex streets” that interact with each other either attractively, in which case clots are generated, or repulsive, in which case these clots are missing.

**Fig. 9.5 (a, b, c, d).** Normalized velocity field for various degrees of fractality:

- a) $x = 0.55$
- b) $x = 1$
- c) $x = 1.6$
- d) $x = 3$

**Fig. 9.6 (a, b, c, d).** Normalized velocity field for various degrees of fractality:

- a) $x = 0.55$
- b) $x = 1$
- c) $x = 1.6$
- d) $x = 3$
We present in figures 9.7 (a, b, c, d) the field of normalized minimum vortices for various degrees of fractality: a) $x = 0.55$, b) $x = 1$, c) $x = 1.6$, d) $x = 3$.

Analyzing the nonlinear behaviors of the minimum vortex field shown in Fig. 9.7 (a, b, c, d) it results that the increase of the degree of fractality, situation explained by the increase of the interactions between the blood entities, implies a decrease of the amplitude of the minimum vortex field as the X and Y spatial coordinates increase.

To perform the experiment we used our biological product (blood) taken in coagulation vacutainers (blue plug); as well as in vacutainer with red plug (without anticoagulant), the latter being necessary for obtaining a smaller clot, which has been introduced in the coagulation sample (fig. 9.3 a, b, c). The patient’s consent to use the collected sample in order to determine the ESR test was not necessary, because the identification of the clot was performed pre-analytically, representing a source of error and implicitly, a non-conformity that is explained verbatim with the recommendation to be repeated sampling.
For the first experiment, we used a sample taken to determine the ESR (erythrocyte sedimentation rate) test in which the macroscopic clot was identified by analysis (tilt maneuver) (fig. 9.8).

**Discussions**

*In vivo* blood flow in vessels and heart is composed of bundles of erythrocytes that are characterized by speed of movement and sense, representing the "target", studied using Doppler ultrasonography (Dudea, Badea, 2009). The estimation of the number of erythrocytes through the power of the Doppler signal contributes to the genesis of the Doppler signal (Dudea, Badea, 2009). If the number of erythrocytes is small, the signal has a low intensity, is finely and imprecisely drawn, as described in fig. 9.11. (Dudea, Badea, 2009). In the situation where a large number of erythrocytes contribute to the genesis of the signal, the same flow, which has the same speed, direction, and character, can be represented by an intense Doppler signal, illustrated in fig. 9.14.b (Dudea, Badea, 2009). These images represent primary information, from which secondary information with improved diagnostic value can be derived (Dudea, Badea, 2009).
Exploration of blood coagulation patterns and rapid detection techniques have attracted attention due to increased demands for rapid assessment of patients in intensive care units, surgery, and other sectors (Wang et al., 2019). In 2005, magneto-elastic transduction was used to monitor and detect changes in viscosity during biological reactions of fibrinolysis and coagulation, being considered a viable option for monitoring processes that are essential for maintaining hemostasis (Puckett et al., 2005).

Free oscillation rheometry is the technique used in a study for the first time, in order to evaluate patients likely to have hypocoagulability and hypercoagulability in pregnant women (Tynngård et al., 2008).

Recent advances in device miniaturization have led to the development of the MHRM device (mHemoRetractoMeter) which uses small amounts of whole blood, being able to measure in real-time, the forces of withdrawal of dynamic clots (Li et al., 2016). For health monitoring and routine diagnosis of hemostatic status, Chen and co-workers presented in their paper, bulk acoustic resonator with a microelectromechanical film that has the advantages of low cost, small size and weight, low sample consumption, and simple operation (Chen et al., 2017).

In 2018, Li and colleagues presented a miniaturized blood clotting test device, by measuring the dynamic development of clot retraction force, which uses carbon nanotube stem sensors that have great potential as a care point for future monitoring of coagulation (Li et al., 2018).

On the other hand, the study of Wang and colleagues aimed to provide information on the core technology for the development of a test instrument, based on electromagnetic induction, which could meet the accuracy requirements of clinical detection (Wang et al., 2019).

Starting from previous studies in which the potential of a US device in vitro with a frequency higher than 20 MHz was presented, to describe the whole blood coagulation process (Ossant et al., 2004, Libgot et al., 2005, Libgot-Calle et al., 2008), Callé and co-workers considered that based on these data, additional information could also be provided about fibrin polymerization, which is an important part of the coagulation process (Callé et al., 2009).

The authors presented a typical ultrasound image during blood coagulation (Fig. 9.15) indicating that clot formation is the result of blood transformation into a solid gel, as a result of the cessation of fluctuations in ultrasonic dissemination after about 2200 s (Huang et al., 2005).
Starting from these images and the block diagram of a Doppler device (fig. 9.16) (Dudea, Badea, 2009), we aimed to develop a miniaturized prototype for the detection of the clot with the US in the sample (OSIM 310782).

**Conclusions**

Based on the experimental results obtained by us, and taking into account the mathematical model presented, we can say that they provide additional motivation for the development of an ultrasound imaging device, so we can evaluate the quality of the samples collected on an anticoagulant to determine coagulation tests. ESR, and in general determinations that may negatively interfere with the presence of clot in vitro, can be considered a valuable non-invasive method.

The use of this device in laboratories ensures analytical performance in terms of guaranteeing the most accurate laboratory results for patients before they are communicated to clinicians.
CHAPTER X

CHRONOMETRIC HYPERCOAGULABILITY - THE PROCOAGULATING ROLE OF TISSUE FACTOR IN VITRO

Introduction
One of the important sources of error for hemostasis determinations is the use of the tourniquet, widely used to define the sampling vein (Nikolac et al., 2013), and which ensures safe venipuncture. Prolonged stasis of the vein associated with damage of the vascular endothelium during venipuncture, accidentally promotes the release of tissue factor (Hernaningsih, Akualing, 2017) historically known as "tissue thromboplastin", with a procoagulant role, both in vivo and in vitro.

Factors involved in the formation of prothrombin from phase II and fibrin during phase III occur in about 15" and do not play a decisive role in the genesis of hypercoagulability, defined as "chronometric hypercoagulability" (Kondi, 1981).

Objectives
The main objective regarded follow up of all shortened PT tests over a certain period and identification of any potential cause-effect relationship between the accidental presence of exogenous FT in vitro with PT determination, that could cause a false shortening.

Material and methods
The present study has been carried out over approximately 4 months, between May and September, on a total of 23615 coagulation samples. All the samples referred to the Hematology Laboratory within the "St. Spiridon" County Emergency Hospital during the interval were collected and analyzed prospectively.

All data were analyzed with SPSS v.24 (IBM SPSS Statistics). Depending on the characteristics of the series of values, the statistical reference indicators were presented (average, 95% CI for average, standard deviation, min, max, etc.).

Results
Before starting of the study, over approximately 4 months we identified all shortened tests of a total of 23615 coagulation samples. There were 544 tests with shortened results indicating a state of hypercoagulability. These were included in the study, meeting the criteria for inclusion in the group. The interpretation of the results was performed by framing the PT values in the normal reference interval (seconds):

a) normal → (10 "-14");
b) shortening / hypercoagulability (<10 ").

To determine the potential in vitro impact of TF on the inaccuracy of 544 PT tests corresponding to the 544 patients included in the study, two sets of determinations were carried out:

a) first determination: reduced PT values
b) second determination: corrected PTs, which had the same shortened value after repeated determination in the primary sample, without recommending repeat sampling (fig. 10.1).
Figure 10.1. Mean PT values depending on the time of determination

Figure 10.2. Histogram of PT values depending on the time of determination

Table 10.2. Evaluation of hypercoagulability according to the second determination

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of tests</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT ≥ 10&quot;</td>
<td>200</td>
<td>36.76</td>
</tr>
<tr>
<td>PT &lt; 10 (hypercoagulability)</td>
<td>344</td>
<td>63.23</td>
</tr>
<tr>
<td>Unchanged PT value</td>
<td>92</td>
<td>16.91</td>
</tr>
<tr>
<td>Modification of the value PT</td>
<td>452</td>
<td>83.08</td>
</tr>
<tr>
<td>Decrease of PT values</td>
<td>102</td>
<td>18.75</td>
</tr>
<tr>
<td>Increasing of PT values</td>
<td>350</td>
<td>64.33</td>
</tr>
</tbody>
</table>
Figure 10.4. Mean values of the differences between the first and second determinations

Figure 10.9. Quantitative evaluation of PT test changes in the presence of clot in erythrocyte sediment

Figure 10.12. ROC curve on the assessment of hypercoagulability for the first determination
Discussions

The permanent transparent and systematic supervision of the testing process encourages and promotes investigations when errors occur, helping to identify procedures and strategies to improve it (Sciacovelli, Plebani, 2009, Agarwal et al., 2012).

Based on previous studies, we assumed that the accidental presence in vitro could cause a false shortening of the PT test. The possible effects of in vitro TF on thrombin generation (FIIa), for the shortened PT test, were assessed by repeating the measurement in the same sample after completion of the first determination.

Based on these findings, we intend to continue the study, to expand the database and analyse in detail the mechanisms of chronometric and structural hypercoagulability, which will cut down the estimation error and results accuracy, as well.

Finally, laboratory specialists are responsible for the reliability of the issued results, given that a false negative or positive result can lead to more expensive procedures and inevitably repeated examinations, not to mention the discomfort created to the patient (Kopec et al., 2003, Englezopoulou et al., 2016). The general principle of medical ethics is based on the good condition of the patient, and a management based on quality aids to increase his satisfaction. The accountability of medical staff is an important pawn in providing medical services that must meet the requirements, have confidence and reduce the frequency of errors in the pre-analytical phase.

Conclusions

We expect these findings to contribute to the interpretation of the curtail results, particularly for the PT test. The accidental presence of TF with procoagulant role in vitro, is decisive for obtaining normal values of the studied parameters.

Our findings support duplication of the determination in the same sample, which could significantly influence the accuracy of the PT test. It is also of interest to laboratory specialists, who are responsible for accurate and precise results delivery, ensuring proper management of patients.

Our study to provide clinicians with key issues in the results interpretation, justifying the need for repetitive, confirmatory, and follow-up tests.
CHAPTER XI

QUALITY OF COLLECTED BIOLOGICAL SPECIMENS – THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS

Introduction

In 2000, Carraro and colleagues pointed out that out of the total number of nonconformities, 60% of the rejected specimens were hemolysed (Carraro et al., 2000). Hence, concerning transmittance of the results it is suggested that clinicians should be aware about this, in order to exclude an in vivo hemolysis (2). In 2006, US pathologists recommended additional investigations for samples with haemolysed plasma, for identifying possible in vivo hemolysis (CAP, 2006). In the same year, Lippi and colleagues concluded that in the case of in vivo hemolysis, in vitro hemolysis is difficile to be avoided (Lippi et al., 2006, OPTMQ, 2008).

Objectives

The aim of our substudy is to shed some light on the effect of hemolysis upon the in vitro coagulation mechanism. The main objective is thus identification of the recommendations resulting from the literature based on the paraclinical studies, in order to adopt the appropriate conduct in relation to performing haemostasis determinations, from the haemolysed samples.

Materials and methods

The progress of the current literature review was performed using the PubMed, Google Scholar and Cochrane database records, based on the following keywords: pre-analytical, post-analytical, hemostasis, hemolysis, incidence, hemoglobin, in vivo, in vitro, results, shortened, PT, APTT, rejected, controversy, investigations, malpractice, comments, information, diagram, visual, clinician, clot.

In order to understand the in vitro mechanism, we scanned for more recent, peer-reviewed original studies describing the effect of the in vitro hemolysis on PT coagulation and APTT coagulation determinations. Study protocols, reported cases as well as opinion polls have been considered.

It was not necessary to obtain informed consent, as the research was based on the cases and protocols presented by colleagues in the literature, which describe the incidence and effect of hemolysis in the context of coagulation determinations.

Results

This review has some limitations given the small number of papers that were referred. Despite this fact, the selected articles can be considered reliable as they describe the samples and the methods used according to the scientific criteria. Being accepted for publication, the selected studies shed some light upon the understanding of the effect of in vitro hemolysis on the determination of PT and APTT coagulation tests. After the initial review, the data were abstracted using a table and have summarized: the interpretation of hemolysis, as well as the mechanism of induction of in vitro hemolysis used by some authors, as described in table 11. 1.
Table 11.1. Relevant studies included

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hernaningsih Y, Akualing JS. (1)</td>
<td>2017</td>
<td>The effects of hemolysis on plasma prothrombin time and activated partial thromboplastin time tests using photo-optical method.</td>
</tr>
<tr>
<td>Arora S, Kolte S, Dhupia J. (9)</td>
<td>2014</td>
<td>Hemolyzed Samples Should be Processed for Coagulation Studies: The Study of Hemolysis Effects on Coagulation Parameters.</td>
</tr>
<tr>
<td>Laga AC, Cheves TA, Sweeney JD. (17)</td>
<td>2006</td>
<td>The effect of specimen hemolysis on coagulation test results.</td>
</tr>
<tr>
<td>Woolley A, Golmard JL, Kitchen S. (20)</td>
<td>2016</td>
<td>Effects of haemolysis, icterus and lipaemia on coagulation tests as performed on Stago STA-Compact-Max analyser.</td>
</tr>
</tbody>
</table>

On the other hand, relevant studies which present the hemolysis colour chart, as well as the interpretation of the hemolysis degree depending on the haemoglobin concentration in the supernatant have been identified (Table 11.2).

Table 11.2. Policies and recommendations regarding the interpretation of hemolysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Title</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binzari E, Zaharia M, Barbu S, Oprea OR, Dobreanu M. (30)</td>
<td>2019</td>
<td>Hemolysis has no influence on routine coagulation tests in subjects without anticoagulant therapy - a referral Romanian emergency hospital laboratory experience</td>
<td>- hemoglobin concentration</td>
</tr>
<tr>
<td>Woolley A, Golmard JL, Kitchen S. (20)</td>
<td>2016</td>
<td>Effects of haemolysis, icterus and lipaemia on coagulation tests as performed on Stago STA-Compact-Max analyser</td>
<td>- hemoglobin concentration</td>
</tr>
<tr>
<td>Arora S, Kolte S, Dhupia J. (9)</td>
<td>2014</td>
<td>Hemolyzed Samples Should be Processed for Coagulation Studies: The Study of Hemolysis Effects on Coagulation Parameters</td>
<td>- color chart</td>
</tr>
<tr>
<td>Plumhoff EA, Masoner D, Dale JD. (28)</td>
<td>2013</td>
<td>Preanalytic laboratory errors: Identification and prevention</td>
<td>- color chart - hemoglobin concentration</td>
</tr>
<tr>
<td>Zhao J, Kan Q, Wen J, Li Y, Sheng Y, Yang L et al. (27)</td>
<td>2012</td>
<td>Hemolysis of Blood Samples has no Significant Impact on the Results of Pharmacokinetic</td>
<td>- color chart - hemoglobin concentration</td>
</tr>
</tbody>
</table>
Discussions

The incidence of haemolysed plasma is common in medical laboratories performing coagulation tests, being a reason for refusing the determinations based on these samples (Favaloro et al., 2012, Freitas, 2015, Hernaningsih, Akualing, 2017).

During our research, we found studies in which the authors triggered the *in vitro* hemolysis by aspirating 1 ml of blood from the primary sample, followed by strong expulsion in the tube through a 23 G needle (Arora et al., 2014).

The experiment was repeated by Hernaningsih and Akualing who used 3ml of blood aspirated in a syringe, creating thus hemolysis by expulsion. This operation was performed twice or thrice (Hernaningsih, Akualing, 2017).

We also referred to studies involving macroscopically identified hemolysis specimens (Laga et al., 2016), as well as those in which the *in vitro* haemoglobin concentration has been measured (Carraro et al., 2000).

As the mechanism of shortening the test in the hemolysed samples was not constant, it was correlated with the possible release of intracellular substances from leukocytes, of phospholipids from erythrocytes or with platelet-induced activation of coagulation (Garton, Larsen, 1972, Freitas, 2015).

Among the literature data, we identified a study by Favaloro et al. that describes the decrease of PT and fibrinogen level following the activation of the coagulation cascade (Favaloro et al., 2012). The APTT prolongation or shortening test is influenced by the fibrinogen loss or fibrinogen activation (Favaloro et al., 2012).

The laboratory should inform the clinician upon the possibility of coagulation investigation tests interference with hemolysis, because the orientation may be different: it may draw the attention of the physician to complete the investigations for a proper diagnosis of hemolytic anemia (reticulocytes, total and direct bilirubin, lactate dehydrogenase, haptoglobin, peripheral blood smear), especially in case of a mild anemic syndrome without clinically significant changes.

Although previous studies recommend rejecting these samples, in our laboratory, we determine PT and APTT tests from slightly hemolysed samples and the results are accompanied by a textual explanation, being in agreement with Carraro and collaborators, attracting the attention (Carraro et al., 2000) of hemolysis possible interference: “Sample with slightly hemolysed plasma, possible interference with the determination”.

In agreement with Magnette's study, by educating the medical personnel involved in the biological product sampling process, we can help reduce, understand and raise awareness of the effects of pre-analytical variables on the coherence and reliability of coagulation screening tests (Magnette et al. 2016).

Conclusions

Considering all the mentioned aspects, a link between the medical laboratory and clinicians should be issued, in order to exclude any *in vivo* hemolysis that could be disregarded as a result of the rejection of samples with haemolysed plasma.

Performing routine screening tests from specimens with a certain degree of hemolysis is accepted, and clinicians may receive reports about the outcomes that fall within the normal reference range.

For patients that follow anticoagulant therapy, the results should be reported with caution, and the possible interference of hemolysis with the determination need to be explained.
CHAPTER XII

MEDICAL STAFF TRAINING - QUALITY INITIATIVE IN REDUCING ERRORS IN THE PRE-PREANALYTICAL PHASE

Introduction
An important role in the modern medicine is played by the laboratory through the quality of the released results, which are essential for the clinicians to establish or exclude a pathology, to accomplish the integrated clinical picture of the patient and, last but not least, to initiate an adequate treatment plan (Crous, Armstrong, 2016). Concerning the haemostasis assays, the reduction of analytical errors is guaranteed through measures ensuring the quality as a result of the use of modern laboratory analyzers (Favaloro et al., 2012).

Objectives
The main purpose of our retrospective study is to evaluate the frequency of the pre-pre-analytical errors in order to quantify the post-analytical performance in the testing process, while relying ourselves on the information and training program for the medical nurses in our hospital clinical departments with the aim to reduce their incidence.

Material and method
Our observational study was carried out over a period of 4 months (June-September) in the Department of Haematology of the "St. Spiridon" Hospital of Iasi. Ethical approval was not necessary, as our research did not aim any interaction with the patient. The objective was to identify nonconforming samples and to create a laboratory policy in order to reduce the error sources in the pre-pre-analytical phase, which otherwise interfere with the detection of haemostasis tests, based on the clinical departments medical staff training. Pre-pre-analytical variables that were recorded as reference data included criteria such as:
- insufficiently collected volume (less than 90% of the required volume);
- haemolysed plasma;
- pre-analytically identified coagulated samples and post-analytically identified specimens with clot in the sediment (Figure 12.1).

Figure 12.1. Description of possible sources of error that could interfere the lab assays
Results
During the 4 months of study, out of the total of 24670 samples taken for carrying out coagulation determinations, 978 (3.96%) nonconforming samples as described in figure 12.3 were introduced into the study, while respecting the criteria for inclusion in the batch presented either insufficiently sampled volume, haemolysed plasma, identified post-centrifugation, totally coagulated or partially coagulated samples (clot evidence).

Figure 12.3. Incidence of nonconforming coagulation specimens

Figure 12.4. Frequency of coagulated samples identified before centrifugation and postanalytically
Table 12.5. Description of non-conformities by clinical sections, prior to and post-training

<table>
<thead>
<tr>
<th>Non-conforming</th>
<th>EU</th>
<th>ICU</th>
<th>Surgical Departments</th>
<th>Medical Departments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficiently collected</td>
<td>1.80%</td>
<td>3.78%</td>
<td>1.26%</td>
<td>1.80%</td>
</tr>
<tr>
<td>Haemolysed</td>
<td>4.50%</td>
<td>14.89%</td>
<td>1.44%</td>
<td>3.30%</td>
</tr>
<tr>
<td>Totally coagulated</td>
<td>10.63%</td>
<td>8.74%</td>
<td>10.63%</td>
<td>8.74%</td>
</tr>
</tbody>
</table>

Discussions

Although the global estimate of laboratory errors is modest, in the case of the haemostasis testing process, the error sources from the pre-analytical phase negatively affect the quality of laboratory results, providing important premises for erroneous diagnosis, equally leading to adverse clinical events that could translate into actual patient illness if not properly identified prior to the report of the results (Plebani, Lippi, 2010, Favaloro et al., 2012, Al-Ghaithi et al., 2017).

The increased fluctuation rate of staff in the medical institutions and, the lack of understanding of the best laboratory practice and inadequate training are all causes of errors in the laboratory results, through inadequate collection of specimens for testing (Lee et al., 2016, Shaikh, Moiz, 2016).

Since training materials and quality control procedures do not include information on possible sources of error it is desirable that the newly hired or non-experienced medical staff coming from different educational environments and with poor background to benefit from continuous monitoring and training in order to reduce nonconformities, maintaining thus the quality outcomes (Lima-Oliveira et al., 2012, Al-Ghaithie et al., 2017).

Although there was no absolute reduction in non-conforming samples at baseline, the main contribution of this study allowed us to test whether training of healthcare staff in the workplace had an effect on the best sampling practice, which is still an important step towards the general reduction of this type of errors in the clinical departments.

Based on the results of this study, certified courses for general and laboratory nurses were organized in collaboration with the professional nurses’ organization OAMGMAMR (Order of General Medical Assistants, Midwives and Nurses in Romania) in 2016, 2017 and 2018. Moreover, starting from 2015 and continuing in 2017 and 2019, informative articles in the organization’s journal publication have been issued, so that the medical staff can actively and continuously learn about the error sources from the pre-pre-analytical phase and their negative implications within haemostasis tests outcomes, for controlling the monitoring of anti-coagulant therapy, and last but not least for patients’ health, thus enabling continuous improvement of medical practices, in agreement with other researchers in the field (Giménez-Marín et al, 2014).

This pilot study was the basis for widening the information activities upon the negative effects of the errors in the pre-pre-analytical phase among clinicians and clinical laboratory specialists, by organizing postgraduate courses which were also offered through continuous medical education system ruled out at the University of Medicine and Pharmacy of Iasi, starting in 2018 and continuing in 2019 and further.

Although it is unrealistic to consider that all critical errors are recorded, learning from mistakes based on reports of nonconformities is also essential (Giménez-Marín et al., 2014).
Conclusions

The haemostasis tests in the haematology laboratory constitute an integral part of the decision-making process, and the false results of laboratory analyses often affect the diagnostic and medical or surgical therapy.

The preanalytical monitoring difficulties beyond the control or direct supervision of laboratory staff require thus effective educational and preventive policies, directed primarily to the medical staff in clinical departments.

These findings highlight the need to improve the sampling techniques standardization, along with the dissemination of operational guides, continuous education, and certification and training of health professionals with responsibilities in the collection and management of biological fluids. This would allow increase in the chance of obtaining high quality accurate specimens, and consistent financial savings for the budget of hospitals, the health system and, last but not least, the patient’s safety.
CHAPTER XIII
QUALITY OF COLLECTED BIOLOGICAL SPECIMENS - THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS

Introduction
In agreement with the international specialized literature, and in the national literature, among the causes of rejection of the samples taken for the determination of PT and APTT (activated partial thromboplastin time) tests, are mentioned: insufficient volume taken, less than 90% (0,5 ml blood less) of the recommended volume (4.5 ml blood), intensely hemolysed plasma and the whole coagulated sample, as described in figure 13.1 (SLMSG, 2007-2008).

Fig. 13.1. Representation of the causes of rejection of coagulation specimens

Objectives
The purpose of this study is to inform clinicians about the negative interference of error sources from the pre-analytical phase, with the determination of coagulation tests, to better understand the possible discrepancies between the results released by the laboratory during dosing and the monitoring of anticoagulant therapies. Emphasizing the need for educational activities in order to reduce the incidence of pre-analytical errors that generate non-compliant blood samples.

Material and method
To investigate the topics of interest, the review of the specialized literature was chosen as a means of using the existing data. The use of evidence-based primary sources, which used different methodologies and theoretical comments on the effect of preanalytical factors on the samples taken to determine hemostasis tests, was desired.

A search on PubMed and Google Scholar was performed to make a comprehensive approach to the negative impact of pre-analytical error sources on the determination of PT and APTT coagulation tests.
Results
In our research, we were able to introduce studies that analyzed the changes occurred during the test process to determine PT test and APTT in the hemolysate samples, with insufficient volume taken and the accidental presence of the clot in erythrocyte sediment.

The selection of the most recent national and international studies (2014-2019) was abstracted using a table, starting from the design mode, objectives, methods of evaluation, sample size and conclusions; implicitly based on the keywords: pre-analytical, post-analytical, coagulation, errors, hemolysis, volume, clot, procedure, false, rejected, education, patient, clinician, nurse, laboratory medicine, trust; as described in Table 13.1.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watson ID, Wilkie P, Hannan A, Beastall GH.</td>
<td>2018</td>
<td>Role of laboratory medicine in collaborative healthcare.</td>
</tr>
<tr>
<td>Hernaningsih Y, Akueling JS.</td>
<td>2017</td>
<td>The effects of hemolysis on plasma prothrombin time and activated partial thromboplastin time tests using photo-optical method.</td>
</tr>
<tr>
<td>Geffen Y, Zaidise I.</td>
<td>2017</td>
<td>At the crossroads: the role of laboratory medicine in the patient care process.</td>
</tr>
<tr>
<td>Woolley A, Golmard JL, Kitchen S.</td>
<td>2016</td>
<td>Effects of haemolysis, icterus and lipaemia on coagulation tests as performed on Stago STA-Compact-Max analyser.</td>
</tr>
<tr>
<td>Freitas F.</td>
<td>2015</td>
<td>What's new about sample quality in routine coagulation testing?</td>
</tr>
<tr>
<td>David RE, Delianu C, Dobreanu M.</td>
<td>2015</td>
<td>Preanalytical errors in Clinic Laboratory Management.</td>
</tr>
</tbody>
</table>
Discussion

Prolonged stasis due to the tourniquet during a difficult venipuncture, along with endothelial damage by puncture, also causes damage to erythrocytes, leukocytes and last but not least platelets (Fig. 13.2), inducing substances with procoagulant activity similar to thromboplastin release, compromising the in vitro quality of the collected sample as a result of significant biological interferences and effects, even if analytically the effects on mechanical systems are negligible (Lippi et al., 2013).

![Biological Interference Diagram](image)

**Fig. 13.2. Analytical and biological interferences due to endothelial, erythrocyte, leukocyte and platelet damage**

Some researchers have estimated that between 9 and 15% of errors are the result of misdiagnosis, with a significant impact on patient care, the likelihood of inadequate care ranging between 2-7% in such cases (Plebani, 2006, Favaloro et al, 2012). Errors resulting from inadequate sampling associated with improper handling and processing of samples will reflect the condition of the collected sample and will not accurately reflect the actual clinical condition of the investigated patient, being considered preanalytical variables (Favaloro et al, 2012).

For patient safety, nurses need to be aware of the influence and possible consequences of preanalytical errors on laboratory results (Makitalo, Liikanen, 2013). Recognition of these aspects represent a real challenge for assistants who have an important role in handling and sampling, an activity that should be considered as a necessary skill, and not just a technical protocol (Makitalo, Liikanen, 2013).
Training of phlebotomy specialists is either a challenge in reducing adverse events that may occur in the investigation of patients, the laboratory having the responsibility for the provision of all necessary information on good sampling practices (GFHT, 2015, Magnette et al., 2016, van Dongen-Lases et al., 2016).

The medical care centrally targeting the patient, is widely accepted and brings novelty in terms of responsibility and medical assistance delivery (Watson et al., 2018). Laboratory medicine through a tripartite collaboration with clinicians and patients who are beneficiaries of these services, must include this change (Watson et al., 2018).

An important step in reducing errors in the pre-analytical phase is also the settlement of a relationship between laboratory and clinic, permanent and mutually beneficial, of relevant information to clinicians on errors that have as a starting point the sampling of the biological product, thus guaranteeing the best results for the patient in the post-analytical phase, as described in fig. 13.3 (Armstrong et al., 2011; Favaloro et al., 2012; Magnette et al., 2016).

![Diagram](image.png)

**Fig. 13.3. Sampling of the biological product "Dark side of the moon" in the diagnostic process**

The best approach in completing the unknown aspects in medical practice is represented by the formation of multidisciplinary teams that include nurses, clinicians, representatives of patients and in vitro diagnostic companies, who constantly collaborate with laboratory practitioners (doctors, biologists, biochemists) to widely disseminate evidence-based recommendations on biological product collection and management, thus initiating corrective measures to improve the quality of laboratory services provided to clinicians and the general population (Lippi et al., 2016).

Highlighting the importance of these errors is necessary for the clinician who, in the pre-analytical phase, decides the tests to be recommended based on the laboratory results received, following a post post-analytical decision on the appropriate treatment (Zemlin, 2018). These aspects are supported by the literature, based on diagnostic errors associated with erosive tests (Epner et al., 2013, Plebani et al., 2014).

**Conclusions**

The seriousness of the potential consequences in the case of false results, generated by error sources interference with the coagulation tests determinations, can be particularly
serious because, in the case of specialized hemostasis tests, these analyzes are often considered "diagnostic".

That’s why we consider to be very important identifying error sources before the results are released to the clinician and before they can turn into a real harm for the patient's life.

Settling a permanent link between clinicians and laboratory specialists would be useful by combining the unique talent of knowing the physiological reason behind the tests and quality laboratory analyzes performance.
CHAPTER XIV

GENERAL CONCLUSIONS

1. The increase in the sensitivity and specificity of clot detection in samples starts from the premise that:
   - a false-positive result could place the patient towards a false diagnosis of hemorrhagic diathesis;
   - a real coagulability disorder could be missed based on a false negative result;
   - a normal false result can place a patient at an unjustified risk of bleeding.

2. It remains a challenge for each laboratory to be able to establish its own procedure for pre-analytical and post-analytical identification of the accidental presence of clot in the sample taken for coagulation determinations.

3. Our experimental results demonstrate the feasibility of using the ultrasonic parameter for in vitro clot detection, which can be exploited to provide the basic technology for the development of a device for this purpose.

4. This prototype can become a promising tool for the miniaturized and automated analytical system that could exclude clots from the samples collected in order to determine coagulation tests, samples that are widely used for routine diagnosis of hemostatic status and personal health monitoring.

5. The accidental presence of the tissue factor with a procoagulant role in vitro is decisive for obtaining normal values of the studied parameters. Our findings support the repetition of the determination of the shortened PT test, from the same sample, with significantly influence upon the accuracy of the result.

6. It is accepted to perform routine screening tests on samples with a certain degree of hemolysis, and the results that fall within the normal reference range can be reported to clinicians. If patients receive anticoagulant therapy, the results should be reported with caution, and the clinician should be informed, explaining the possible interference of hemolysis with the determination, once the results have been issued.

7. Our study demonstrates the need to improve the standardization of sampling techniques along with the dissemination of operational guidelines, continuing education, certification, and training of health professionals with responsibilities in the collection and management of biological fluids.

8. Establishing a permanent link between clinicians and laboratory specialists (doctor, biologist, biochemist) would be useful by combining the unique talent of knowing the physiological rationale behind the tests and quality laboratory analyzes performance.

9. The evaluation of the quality of medical services in laboratories has a great significance, with serious implications in the management of laboratory tests, which, especially in the case of hemostasis tests, are often considered "diagnosis"; In addition, identifying the sources of error before the results are released to the clinician is imperative for the correct management of the patient's condition.
OPEN PERSPECTIVES OF THESIS RESEARCH

Laboratory medicine plays a key role in the provision of medical services, as laboratory tests are a cornerstone in the effective provision of all necessary information to clinicians in order to make correct medical decisions, according to the real biological condition of the patient. Patient-centered care is gaining international acceptance, assuming greater responsibility for its health, a permanent, tripartite collaboration between patient-clinician-laboratory being mandatory so that accountability and delivery of medical services change radically in favor of the patient.

The main purpose of this doctoral thesis was to contribute to the development of laboratory medicine, by identifying, testing, and implementing procedures to ensure the effectiveness of results, in accordance with those used at the international level. By carrying out this manuscript, we aimed to evaluate the prevalence of pre-analytical (clinical) sources of error; for this, biological fluid has been sampled to determine coagulation tests, in order to update the standards for assessing the compatibility of results released by the laboratory, so as to create a real database, starting with the sampling of the biological product from the clinical departments.

Pre-analytical errors in the clinic can translate during diagnostic laboratory investigations into situations that lead to increased patient morbidity and mortality. In this regard, the results of our research draw attention to knowledge-based healthcare, highlighting the effect of these sources of error on the determination of coagulation tests, as well as the role of educational care activities on the incidence of pre-analytical errors, which generated these non-conforming samples.

Failure to identify inappropriate specimens, in time can have particularly serious consequences that endanger the patient's life, including unnecessary treatments and tests, as well as increased hospitalization periods, which ultimately lead to delays in establishing a correct diagnosis or initiating treatment.

The elements of originality brought by this study are also conferred by the post-analytical in vitro clot procedures used, and by their correlation with the effectiveness of the results issued by the laboratory, in order to support the therapeutic procedures to be implemented by clinicians. They are also basic contributors to the development of an innovative device that uses ultrasound to detect clots in the total volume of collected blood.

This study has many promising results and opens up new research perspectives:

- characterization of the profile of all sources of error identified pre- and postanalytical in the samples taken on different anticoagulants to highlight the interferences with the determination of the tests;
- elaboration of a set of pre- and postanalytic investigations, focused on the use of available tests for evaluation of the levels of compatibility between the obtained results and the real biological condition of the patient;
- elaboration and implementation in the clinic of some relevant variants of training and permanent information of the medical staff, responsible for the quality of the sampled biological product;
- implementation of a minimum set of tests for the periodic evaluation of nurses in clinical departments, establishing thus the eligibility criteria for their selection in compliance with the conditions recommended by medical laboratories, aiming to release high accuracy results;
- analysis of the risks of issuing false results in anticoagulant therapy and elaboration of a long-term post-analytical surveillance program;
- analysis of the incompatibility of the results obtained from the samples identified with pre- and postanalytic error sources generated by the lack of information of clinicians, which in turn are responsible for coagulation tests recommendation, clinical picture completion, diagnosis and anticoagulant therapies monitoring, that must be free of bleeding risk for the patient.
SELECTIVE REFERENCES:


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