Diagnostic Challenges in Newborns and Infants with Coagulation Disorders

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Keywords
- coagulation diagnostics
- infant
- preterm
- newborn

Abstract
Laboratory diagnostics in children and adolescents, especially in newborns and very small children, differ considerably compared with laboratory diagnostics in adults. This applies to all individual steps of the examination (i.e., the indication, the preanalytical phase, the analysis itself, and interpretation of the findings). This is particularly true in the diagnostics of hemostasis, in which small sample volumes and relatively error prone coagulation tests are posing particular challenges to the strategy, performance, and evaluation of the tests. Differences in the individual steps are illustrated below.

Introduction
The clinical problems that today lead to presentation of patients in an outpatient coagulation clinic vary greatly from age group to age group. In recent years, the main focus in adults has been on clarifying their tendency toward thrombosis, monitoring anticoagulation, and clarifying their tendency toward bleeding. In childhood and adolescence, perioperative coagulation diagnostics and the assessment of an increased bleeding tendency are the most common indication. In recent years, there is also increasing demand in the field of neonatology and intensive care diagnostics especially for catheter-related complications or sepsis-related coagulation disorders. Standard routine parameters are not suitable.1 A premature infant weighing of 1,000 g has an intravascular volume of 100 mL; a newborn infant, approximately 250 mL. This means that the sampling of a standard 5-mL vial represents 2 or 5% of the total blood volume. The first challenge is therefore to limit diagnostics to the bare essentials. Routine screening diagnostics or the creation of “profiles” are therefore obsolete. For instance, in the case of a familial predisposition, there is no indication for a thrombophilia diagnosis in prepubescent children who are not clinically affected by vascular occlusion with the exception of determination of antithrombin III or protein C in families with severe defects. The routine preoperative coagulation diagnostics in infants can almost always be replaced by taking a systematically collected medical history.2 The most important criterion for selecting the particular parameters to be investigated is therefore whether the result has a therapeutic consequence. The ideal diagnostic procedure should be based on the leading symptom with a clear target orientation. – Tables 1 to 3 provide suggestions for frequently asked questions.

Preanalytical Problems
An abundance of possible disruptive factors and sources of error in coagulation diagnostics has long been identified and led to the well-known recommendations for sample

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Technical aspects have a completely different significance in the training of a pediatrician than in other medical staff; it often takes months to years until venipuncture for blood collection is safely mastered, especially in premature and new-born babies. This means that hemolytic samples, underfilled tubes or clotted samples, and even clot formation are rather frequent during blood collection in the coagulation laboratory of a pediatric clinic. The initial quality control by the technical staff and taking notes of the diagnostic limitations are therefore essential for the later interpretation of the results. In a neonatal intensive care unit, hematocrit values between 30 and 70% are not uncommon. This can compromise the measured results; since the plasma:citrate ratio varies from 6.3:1 to 2.7:1 depending on a corresponding “dilution effect.” However, this also implies that underfilled and overfilled samples can still be examined depending on the hematocrit value. For this purpose, we have developed a tool that allows the technician to assess whether the sample can be measured based on a known hematocrit value (►Fig. 1).

In almost all clinics, coagulation diagnostics are performed in central laboratories in which samples of children are practically quantitatively negligible. The selection of devices, tests, and reagents will therefore rarely take into account the specificity of the pediatric needs. However, there are differences in the systems available on the market, especially with regard to the required sample volume. In principle, no major difference needs to be considered when selecting test systems except in the case of derived fibrinogen, which is not suitable for newborns or infants because of poor and inconsistent correlation between prothrombin time-derived fibrinogen determination and the Clauss method. Icteric and lipemic plasma disturb the PTZ-derived fibrinogen determination. An ideal investigation would be a global test that would provide as much information as possible with as little starting material as possible. Unfortunately, the otherwise

### Table 1 Stepwise diagnostic work-up of acute bleeding event

<table>
<thead>
<tr>
<th>Preterm/newborn</th>
<th>Acute bleeding event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary coagulopathy</td>
<td>Acquired coagulopathy</td>
</tr>
<tr>
<td>von Willebrand disease</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Hemophilia A/B</td>
<td>Vitamin K deficiency</td>
</tr>
<tr>
<td>Other factor deficiencies</td>
<td>DIC</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
</tr>
</tbody>
</table>

**Platelet function defects**

| Level 1 | Blood count, PT, aPTT, fibrinogen, F XIII, vWF:Ag |
| Level 2 | Single factors depending on PT/aPTT constellation |
| Level 3 | Platelet function analysis (aggregometry) |

**Therapeutic implications**

- Vitamin K substitution, platelet transfusion, FFP, or single factor infusion

### Table 2 Stepwise diagnostic work-up of neonatal thrombosis or stroke

<table>
<thead>
<tr>
<th>Preterm/newborn</th>
<th>Thrombosis, stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary coagulopathy</td>
<td>Acquired coagulopathy</td>
</tr>
<tr>
<td>Protein C/S deficiency</td>
<td>Polycythemia</td>
</tr>
<tr>
<td>Antithrombin III deficiency</td>
<td>Sepsis/DIC</td>
</tr>
<tr>
<td>Other thrombophilic states</td>
<td>Central line associated</td>
</tr>
<tr>
<td>TTP when additional hemolysis</td>
<td></td>
</tr>
</tbody>
</table>

| Level 1 | Blood count, PT, aPTT, D-dimer, antithrombin III, protein C |
| Level 2 | Single factors depending on PT/aPTT constellation |

**ADAMTS13 activity when hemolysis is present**

**Therapeutic implications**

- Monitoring of anticoagulation, substitution of protein C/antithrombin III

### Table 3 Diagnostic work-up of bleeding symptoms or acute event (infants)

<table>
<thead>
<tr>
<th>Infant</th>
<th>Unusual bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary coagulopathy</td>
<td>Acquired coagulopathy</td>
</tr>
<tr>
<td>von Willebrand disease</td>
<td>Thrombocytopenia</td>
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<td>DIC</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td></td>
</tr>
</tbody>
</table>

**Platelet function defects**

| Level 1 | Blood count, PT, aPTT, fibrinogen, F XIII, vWF:Ag/activity |
| Level 2 | Single factors depending on PT/aPTT constellation |
| Level 3 | Platelet function analysis (aggregometry) |

**Differential diagnosis of thrombocytopenia (blood smear)**

**Therapeutic implications and diagnostic consequences**

- Vitamin K supplementation, factor substitution
- Treatment of thrombocytopenia depending on diagnosis

**Abbreviations:** aPTT, activated thromboplastin time; DIC, disseminated intravascular coagulation; FFP, fresh frozen plasma; PT, prothrombin time.
useful prothrombin time and activated thromboplastin time in childhood are not suitable for preoperative diagnostics, for a reliable diagnosis or for the exclusion of a factor deficiency, because false-positive and false-negative test results are frequent.  In addition, the hope that thrombelastography would be able to remedy this problem has not completely fulfilled, but actually some promising studies in neonatal intensive care units were done. Similarly, the “in vitro bleeding time” (PFA-100 or similar devices) could not be established as a screening method in childhood and adolescence since relevant preanalytical implications, and as well as poor sensitivity and specificity for individual problems limit the diagnostic statement power in pediatrics.

Interpretation of Findings

However, the greatest challenge in diagnostics in this age group is evaluating interpreting the test results obtained. The coagulation system of premature babies, newborns, and infants in the first year of life is subject to a maturation dynamic that requires a differentiated knowledge of age-related reference values. The first publication of such data by Maureen Andrew was a milestone. Several current publications on this topic have appeared in recent years. These also take into account the fact that the technical development from early mechanical detection methods to light-optical methods and above all the development of new parameters now require a more differentiated examination interpretation of the findings. In many publications, it is recommended to establish laboratory reference values that refer to the local conditions of the equipment and the selection of the reagents. However, this requirement quickly would breach ethical limits necessitating the examination of healthy premature and newborns as well as infants. As a rule, it is best to use published reference values that have been created using a similar methodology. However, it is crucial that the connections between the clinical symptoms and, if necessary, the dynamics of the clinical disease course are closely correlated with the laboratory findings. Therefore the clinician and the laboratory manager should maintain a close communication with each other. At this point, it should be mentioned once again that personal and family medical histories can often provide valuable information.

Summary

In newborns and infants, the diagnosis of coagulation disorders (from the indication to the interpretation of the findings) differs in many ways from that of older children or adults. Ideally, a pediatric hemostaseologist can discuss the selection of equipment and test systems in the central laboratory. The interpretation of the findings in intensive care units and in surgery also requires expertise that takes into account the specific characteristics of each age group. In a large hospital, the proportion of coagulation tests for newborns, premature babies, and infants represents only a negligible proportion part of the total workflow volume of a
in the laboratory. Therefore, constant contact between the clinician and the laboratory is mandatory to ensure a high level of quality.

Conflict of Interest
None.

References