was >20 genome-equivalents per milliliter, and the amount of circulating DNA in maternal plasma would be sufficient to analyze fetal gender.

In conclusion, this is the first description of the diagnostic accuracy of fetal gender determination from a large number of maternal plasma samples obtained at early gestation. Because this test is noninvasive and highly accurate, it can be used as a valuable first step in various clinical settings.

This work was supported in part by the High-Technology Research Center Project from the Ministry of Education, Science, Sport and Culture of Japan and by Grants in Aid for Scientific Research from the Ministry of Education, Science, Sport and Culture of Japan (Nos. 11770960, 12770933, 13770941, and 13770940).

References

Cryoglobulins Interfere with Platelet Counts by Optical and Impedance Methods but not with the CD61 Immuno-platelet Count. Nicolas von Ahlsen,1 Bettina Ehrlich,2 Colin Stephen Scott,3 Joachim Riggert,2 and Michael Oellerich1 (Departments of 1 Clinical Chemistry and 2 Transfusion Medicine, Georg-August-University, Robert-Koch-Strasse 40, 37075 Goettingen, Germany; 3 Abbott Diagnostika GmbH, 65205 Wiesbaden-Delkenheim, Germany; 4 author for correspondence: fax 49-551-39-12504, e-mail nahsen@gwdg.de)

Automated complete blood counts (CBCs) use supplementary warnings (flags) to provide alerts for the existence of hematologic abnormalities (1). We observed on several occasions platelet-count interference by cryoglobulins, where the impedance and optical results were in error to a similar extent and did not trigger a delta alert.

Platelets usually are counted by impedance analysis or by optical procedures (refractive and laser light-scattering properties). The CELL-DYN CD4000 system (Abbott Laboratories) simultaneously provides both and offers a further optional approach using immunoplatelet analysis (2, 3). In the latter method, a fluorescein isothiocyanate-labeled monoclonal antibody (CD61; Clone RUU-PL 7F12, IgG1 subclass) binds the glycoprotein (Gp)IIa part of the GpIIb/IIIa receptor subunit (3). This glycoprotein is expressed by all human platelets, both resting and activated, but not by erythrocytes or leukocytes. The immunochromatographic platelet count is largely unaffected by sample interferences and allows determination of platelet counts to <1 × 10^9/L (4). The analytical performance is virtually identical to that reported for alternative flow cytometric immunomethods (5).

We recently encountered four patients with cryoglobulinemia in whom erroneously high platelet counts were obtained by both optical and impedance platelet counts. Patient 1 had lymphoplasmacytoid lymphoma. Because the CD4000 analyzer flagged the presence of variant lymphocytes, a peripheral blood smear was examined. This confirmed the presence of lymphoma cells and also revealed thrombocytopenia, whereas the automated impedance and optical analyses suggested platelet counts of ~355 × 10^9/L (Table 1). The result from a chamber (hemacytometry) count (38 × 10^9/L) also indicated thrombocytopenia, and a CD4000 CD61 immunoplatelet count (42 × 10^9/L) further substantiated the morphologic conclusions. Therefore, both the impedance and optical platelet counts were overestimated and, because the errors were of similar numeric magnitude, no CD4000 delta alert was given. Consequently, this patient’s thrombocytopenia would have been missed had the manual slide review not been performed for other reasons. We processed these same samples with other hematology analyzers (Sysmex SE8000, Sysmex K4500, Sysmex XE2100, and Bayer Technicon H*3) and found platelet count overestimates similar to that of the CD4000.

Patient 2 (Table 1) had Waldenström macroglobulinemia, but at the time of CBC analysis he was not thrombocytopenic. Both the CD4000 (impedance and optical) and Technicon H*3 (optical) instruments significantly overestimated the platelet count. Two other patients seen during the past 18 months demonstrated similar patterns of interference. Both had cryoglobulinemia, one associated with centroblastic lymphoma (patient 3) and the other with Waldenström macroglobulinemia (patient 4; see Table 1). These patients also had several episodes of thrombocytopenia during their hospital stays for cytoreductive chemotherapy.

Because of these observations, we wished to develop a review procedure for recognizing such situations in the absence of a significant difference between CD4000 impedance and optical platelet counts. Frequency distributions of platelet size may show increased steepness or asymmetry in the lower ends of platelet size histograms.
because of smaller-sized non-platelet particles such as cryoglobulin. The Technicon H*3 analyzer displayed a typical peak indicative of cryoglobulinemia (6) in its optical platelet size plot (Fig. 1A; patient 2), but this was not seen in the CD4000 impedance histogram (Fig. 1B). In addition, the CD4000 optical scatter graph (Fig. 1C) did not appear unduly abnormal, although there was some suggestion of a significant accumulation of events at the

<table>
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<th>Patient</th>
<th>Disease</th>
<th>Cryocrit, %</th>
<th>CD4000 optical count</th>
<th>CD4000 impedance count</th>
<th>CD4000 immunoplatelet (CD61) count</th>
<th>Technicon H*3 platelet count</th>
<th>Hemacytometer (chamber) count</th>
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Fig. 1. Platelet distribution for patient 2.

(A), histogram representing platelet size distribution of patient 2 (Table 1), who had cryoglobulinemia as determined with the Technicon H*3 analyzer. The reported platelet count was $819 \times 10^9/L$, although the observed histogram with a high event frequency in the very low size range suggests that it was erroneous. Analysis of the same sample with the CD4000 impedance (B; $578 \times 10^9/L$) and optical (C; $619 \times 10^9/L$) platelet counting methods does not reveal any obvious abnormal distributions, although there appears to be an unusually sharp cutoff at the lower threshold on the $7^{th}$ axis of the optical plot. Subsequent analysis with the CD4000 immunoplatelet method revealed a platelet count that was significantly lower ($226 \times 10^9/L$) than the counts obtained by the other methods. In the immunoplatelet graphic plot (D), a large number of gray events (non-platelet) are seen to overlay the cluster of platelets (black events).
lower end of the 7° axis scatter. Thus, atypical CD4000 platelet plots are not always seen in cryoglobulinemia, although other interferences are usually detected with high efficiency. For comparison, whereas the Technicon H1+3 appeared to provide a more immediate visual indication of sample cryoglobulins, large platelet count overestimates were still obtained in most cases.

Examination of graphic plots is not used in all laboratories, and its efficiency may be limited when workload is high. Without it, operators depend on the presence of other hematologic abnormalities, instrument flags, or clinical information. Because patients with cryoglobulinemia, both at clinical presentation and during treatment, almost always show abnormal erythrocyte or leukocyte results, morphologic review is likely.

When atypical platelet counts are suggested by graphic or morphologic review, a systematic approach to subsequent investigation can be initiated. Initially, warming samples (>30 min at 37 °C) before instrument analysis generally resolves cryoglobulin interference and enables platelet counting by standard optical or impedance methods. When interference persists after rewarming, other causes should be considered.

When doubt remains regarding the true platelet count, the conventional alternative is manual hemacytometry, but its imprecision is unacceptably high when the platelet count is <20 × 10⁹/L. The CD4000 CD61 immunoplatelet count, however, has high precision in thrombocytopenia (4) and, moreover, is not influenced by the presence of cellular or plasma interferences (Fig. 1D). Despite the cost of the test (approximately US $16 in Germany), immunoplatelet counting should be considered in situations such as platelet transusions and therapeutic interventions with bleeding risk.

Few reports have described platelet count interference by cryoglobulins, although awareness of the potential analytical problems is high. Because the presence of thrombocytopenia was masked in two of the four cases described here, we believe that cryoglobulin interference is an important concern for the clinical laboratory.

We thank Dr. Hans Seeger for helpful discussions and Prof. Victor W. Armstrong for valuable editorial support.

References

Quantitative Reverse Transcription-PCR Comparison of Tumor Cell Enrichment Methods, András Ladányi,2*, Richie Soong,2 Karim Tabiti,2 Bela Molnár,1 and Zsolt Tulassay1 (*Semmelweis University, Second Department of Medicine, Szentkirályi u. 46, 1088 Budapest, Hungary; 2 Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany; * author for correspondence: fax 36-1-2660816, e-mail ladus@bel2.sote.hu)

The feasibility of detecting disseminated tumor cells through reverse transcription-PCR (RT-PCR) detection of tissue-specific genetic markers in extra-tumor compartments is currently unclear because of conflicting findings on assay sensitivity and specificity between studies [for reviews, see Refs. (1–3)]. One potential source of these assay discrepancies includes the different sample preparations and analysis methods used between laboratories (4). Many studies have used nonenriched (NE) whole blood, although the high population of background cells within these preparations is considered to reduce RT-PCR sensitivity and specificity (5). To improve sensitivity and specificity, some investigators have used density-gradient enrichment (DGE) that is directed toward separating tumor cells in a mononuclear cell fraction from peripheral blood according to cell density (5, 6). Others have tested immunomagnetic separation (IMS) to isolate tumor cells with antibody-coated magnetic beads directed against epithelial cells (7, 8). The lack of standardized procedures and the paucity of quantitative analysis and of studies examining these methods in parallel have made the extent of the influence of sample-preparation methods difficult to determine.

To assess the effect of sample preparation on the RT-PCR-based detection and quantification of disseminated tumor cells, we studied healthy donor blood samples with added colorectal cells. We used a newly developed, quantitative RT-PCR assay for the putative gastrointestinal epithelia-specific marker cytokeratin 20 (CK20) (9–12) to evaluate the sensitivity, specificity, and quantitative PCR effects of DGE, two IMS techniques, and a NE preparation.

For each series, 80 mL of peripheral blood were drawn from the antecubital vein of healthy volunteers with a Vacuette into heparinized tubes (Greiner). To avoid epithelial cells from skin puncture, the first 5 mL were always discarded. We added, per mL, 1000, 100, 10, 1, and 0 HT29 colorectal carcinoma cells to triplicate 5-mL blood aliquots. The 100 and 1000 cells/mL samples were prepared by dilution and the 10 and 1 cells/mL samples by micromanipulation. Each series was processed by either DGE of peripheral blood mononuclear cells with the Ficoll-Histopaque®-1077 (Sigma), IMS with Epithelial Enrich® Ber-EP4-coated microsize beads (Dynal), IMS with a CellSearch Epithelial Cell Enrichment Kit® and anti-EpCAM-coated nanosize beads (Immunicon), or without enrichment. The dilution series were repeated three times for each method with blood from different healthy donors. This gave 9 preparations (triplicates, 3 donors) of each of the 5-cell concentrations and 45 determinations for