Adenosine Deaminase in Pleural Effusions

To the Editor:
Villena et al. reported on the value of automated determination of adenosine deaminase (ADA) and lysozyme in the differential diagnosis of pleural effusions [1]. Recent findings on ADA should perhaps be considered in discussions. The authors state: "Methods commonly used to quantify ADA and lysozyme are manual and time-consuming; consequently, clinical decisions based on these measurements may occasionally be delayed." However, we previously described an automated method for ADA [2].

Villena et al. refer to the ubiquitous and polymorphic nature of ADA and imply that lymphocytes are the source of increased activity in tuberculous effusions. Two isoenzymes of ADA exist (ADA1 and ADA2), coded for by different gene loci [3]. We have shown that while ADA1 does indeed have an ubiquitous tissue distribution, its highest concentrations being in lymphocytes and monocytes, ADA2 could not be demonstrated in any tissue except monocytes, in which it contributes a small fraction of total ADA activity [4]. An increase of ADA2 in serum was associated with diseases in which the monocyte/macrophage system is actively involved [4]. We also found that ADA1 is the dominant isoenzyme in tuberculous effusions, whereas ADA2 predominates in parainfective and lymphoproliferative disease effusions [5, 6]. If Villena et al. had distinguished between the isoenzymes, the specificity of ADA determination would probably have been better, even if the empyematosus effusions (which had high ADA concentrations) were included in their calculations. In the rheumatoid arthritis cases, which are also characterized by increased monocyte/macrophage activity, one would expect to find an increase in ADA2 activity similar to that found in tuberculous effusions. This has been confirmed in our studies, which showed that in rheumatoid arthritis the increase in ADA activity in sera [4] and effusions (unpublished results) was due to ADA2.

We think that any speculation on the cause of ADA increase should be based on the isoenzyme pattern, given that the origin and characteristics of each are completely different. Different diseases are characterized by unique ADA isoenzyme patterns, depending on the pathophysiology. The determination of isoenzymes should therefore greatly enhance the diagnostic value of ADA.

References

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The authors of the report mentioned reply:
To the Editor:
We read with interest the comments by Ungerer and Bissbort. Indeed, they first reported an automated method to measure ADA concentrations [1]. Their method, based on coupling the enzymes nucleoside phosphorylase (EC 2.4.2.1) and xanthine oxidase (EC 1.1.3.22), was automated for use with a Cobas Mira analyzer. By contrast, we use a kinetic method based on the coupling with a NADH/NAD reaction, automated for a Hitachi 717 analyzer [2]. This latter analyzer allows ADA to be measured simultaneously with the rest of the laboratory analyses used in the differential diagnosis of pleural effusions.

In addition, Ungerer and Bissbort refer to the improvement of ADA specificity by distinguishing between the isoenzymes. It is true that ADA2 is the dominant isoenzyme in tuberculous effusions, whereas ADA1 predominates in parainfective and lymphoproliferative disease effusions. Therefore, if we had distinguished between isoenzymes, parainfective and lymphoproliferative effusions would probably not have been misclassified. In that case the specificity would increase from 95% to 97%, a statistically nonsignificant difference. These data are consistent with those reported recently by Valdés et al. [3] and cast doubt on the contribution of ADA isoenzymes to the diagnosis of pleural effusions.

References

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Errors in Neonatal Bilirubin Measurement

To the Editor:
I read with interest the timely report by Vrman et al. [1] and the editorial by Doumas and Eckfeldt [2] regarding the continued errors in measurement of total bilirubin. As a clinician, I wish to reiterate the importance of accurate measurement of serum bilirubin in the neonatal range because therapeutic interventions depend on the values obtained. Inaccurate values may result in unnecessary blood sampling, increased costs, and potentially life-threatening procedures. Recommendations for pediatricians and neonatologists for appropriate practice parameters for managing hyperbilirubinemia in the healthy term newborn have been published by the American...
Academy of Pediatrics [3]. The success of national practice guidelines will depend on the availability of reasonable and accurate estimates of the serum bilirubin.

The National Academy of Clinical Biochemistry is developing standards of laboratory practice and recommendations for monitoring the status of newborns, in particular for assessing liver function and hyperbilirubinemia. I have been requested to spearhead this section, and these laboratory practice guidelines will be published in a future issue of Clinical Chemistry. Both the laboratory and the clinician are under tremendous pressure to provide cost-effective quality services. The recent policy of discharging newborns earlier from the nursery makes accurate bilirubin determinations in these newborns increasingly important.

The proposed guidelines must take into account the inaccuracy of bilirubin determinations as measured in many of the finest institutions in our country. Collaboration among clinicians, laboratorians, and manufacturers is critical to improving bilirubin standardization for newborn specimens. Refined action guidelines for the care and management of the newborn will result.

References

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Two-Layer Buoyant Density Centrifugation Gradient for Enrichment of Prostate-Derived Cells and Cell Clusters from Peripheral Blood

To the Editor:
Satisfactory clinical management of prostatic cancer requires sensitive markers allowing accurate presurgical staging and the detection of metastasis. The success rate of radical prostatectomy is critically dependent upon restricting the tumor to the prostate gland and is much lower in cases where the tumor has spread to the bone marrow [1–7]. We here report data on the density gradient step of our recently published combined buoyant density gradient immunomagnetic separation method [8], which proved to enrich prostate-derived cells from peripheral blood of patients.

To enrich prostate-derived cells from peripheral blood, we used a two-layer buoyant density gradient. We filled 12-mL polystyrole tubes, saturated with 10 mL/L fetal calf serum in phosphate-buffered saline (PBS) for at least 2 h with 3 mL of PolyomorphPrep™ (d = 1.113 kg/L, osmolality = 460 mOsm/L; BRL, Eggenstein, Germany), and carefully overlaid them with 3 mL of NycoPrep™. 1.068 (d = 1.068 kg/L, osmolality = 335 mOsm/L; BRL). On top of the gradient was laid 5 mL of EDTA-treated blood. The tube was centrifuged at 450g for 20 min at room temperature. The single-cell band between the interface of the platelet-enriched plasma and the infranate with a density of 1.068 kg/L was carefully removed with a small syringe (Fig. 1B, indicated as epithelial cells). This gradient-enrichment method was evaluated with cancer cell lines and disaggregated tissue from benign prostate hyperplasia and prostate cancer as follows: 10^7 cells were incubated with [3H]thymidine for 12 h with gentle agitation in a closed, sterile tube. The cells were washed with PBS and centrifuged at 200g. The pellet was suspended in 1 mL of PBS. Finally, the cells were mixed with 40 mL of EDTA-treated peripheral blood and subjected in 5-mL portions to density gradient centrifugation, as mentioned above. For measurement of the radioactivity, the gradient was transferred in 500-μL portions to scintillation vials containing 5 mL of lysis buffer (75 mmol/L NaCl, 25 mmol/L Na2EDTA, pH 8.0). Scintillation cocktail was then added and beta activity was measured. The total cell numbers before and after density gradient enrichment were counted on a Neubauer (Oberkochen, Germany) hemocytometer. Cells were labeled according to their proliferation activity by using a [3H]thymidine incorporation assay. The results of three experiments for prostate cancer and benign prostate hyperplasia tissues with the buoyant two-step gradient procedure are shown in Fig. 1. The prostate cancer cells (Fig. 1, filled boxes) were separated in a sharp band at the interface between platelet-enriched plasma and the 1.068 density level. Recovery of prostate-derived cells was up to 90% as calculated from the specific radioactivity. The cells from benign prostate hyperplasia showed more density heterogeneity. As seen from Fig. 1 (open circles), the greatest portion of cells was found above and at the interface between platelet-enriched plasma and 1.068 density level, although cells labeled with tritium were also found at higher densities.

To validate the results of the [3H]thymidine-labeling experiments, we performed microscopy on three portions of the buoyant aspirant from centrifuged samples of blood from healthy volunteers, to which had been added prostate cancer cells, and from samples of blood from patients with metastatic prostate cancer. These portions were (a) platelet-enriched plasma, (b) a fraction including the interface between the platelet-enriched plasma and the infranate (the prostate-derived cell-containing fraction), and (c) the lower fraction (d >1.077) containing the lymphocyte and the granulocyte fraction. Prostate-derived cells were sometimes found in fraction (a) but never in fraction (c).

The disaggregated prostate cancer cells are also prepared as cancer cell clusters present in the blood subjected to density gradient separation. Because separation of cells in the density gradient is due to differences in the relation of mass and volume, the density gradient allows the isolation of single prostate cancer cells and cell clusters of similar density.

Prostate cancer cells and cell clusters could be isolated from peripheral blood of prostate cancer patients, as already shown for breast cancer patients [9]. The prostate-derived cells were identified by anticytokeratin antibodies and were visualized with alkaline phosphatase–Newfuchsin reaction (Fig 1C). Our results also agree with those of Ferro et al. [10], who used a four-density-layered gradient to detect proliferating prostate cancer cells after hormonal stimulation and found the proliferating prostate cancer cells in a single band at 1.056 kg/L. Compared with their method, our approach is easier and quicker to perform and has a higher recovery of prostate-derived cells.