redicting both adverse cardiac events and readmissions in congestive heart failure inpatients (5).

Interestingly, as clinicians learned to use BNP results in various clinical settings, it was in the outpatient setting that BNP test usage experienced the most growth in (8% in 2001 to 47% in 2003), accounting for the majority of assays ordered in the time period assessed in 2003. Although monitoring of BNP in the outpatient setting has not yet been shown effective in tailoring treatment of congestive heart failure, several large-scale studies are underway to test this hypothesis. The fact that effective treatments for heart failure have been correlated to decreasing BNP concentrations bodes well for future applications in tailoring treatment and in determining the overall stability of the patient outside the hospital (6). These results may also help delineate the appropriateness of the use of BNP testing in the outpatient setting and avoid any overuse that might occur. BNP concentrations have also been shown to be of value in several outpatient situations, including assessing volume overload and prognosis in renal failure (7), as well as in assessing the severity of valvular heart disease (8).

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References


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 Measurements, Zymographic Analysis, and Characterization of Matrix Metalloproteinase-2 and -9 in Healthy Human Umbilical Cord Blood

To the Editor:

Matrix metalloproteinases (MMPs) are zinc/calcium-dependent effectors/mediators of physiologic and pathologic reproductive processes (1); MMPs may alter fetal homeostasis and be involved in pathologic syndromes of pregnancy (2). No information is available on MMPs circulating in healthy umbilical cord plasma (UCP). To characterize the effect(s) of umbilical cord blood (UCB) sampling and to identify possible cellular source(s) of MMP-2 (EC 3.4.24.24) and MMP-9 (EC 3.4.24.35) circulating in UCB, we investigated their concentrations, biochemical characteristics, and isoform distributions by immunooassay and gelatin zymography.

We collected UCB (n = 20) immediately after delivery from women with uncomplicated healthy pregnancies; mean (SD) gestation age at delivery was 38 (2) weeks. We used plastic tubes containing lithium heparin, dipotassium EDTA, or buffered sodium citrate (9NC); sera were obtained from clot-activator-gel tubes (SST; Becton Dickinson). After centrifugation at 1000g for 15 min, supernatants were collected. Leukocyte subpopulations were tested for their subsets and gelatinase content after sedimentation on Lympholyte® gradient (Cedarlane) (3).

Umbilical cord vein sections (n = 5) were excised, separated from the Wharton’s jelly, homogenized, and centrifuged at 8000g for 30 min; the supernatants were then analyzed (4).

Gelatinase calibrators were prepared from healthy capillary peripheral blood (3). MMP molecular isoforms were analyzed under non-reducing conditions on gelatin-copolymerized polyacrylamide gels (5). Western blotting was performed with anti-human MMP-2 and -9 monoclonal antibodies (clones 75-7F7 and GE-213, respectively; Calbiochem). We measured UCP gelatinases by Biotrak™ MMP-2 and MMP-9 assays (Amersham Pharmacia) (5). Mean (SE) values of three independent experiments performed in duplicate were calculated; statistical analysis was performed with the Student t-test and Mann–Whitney U-test. P values < 0.05 were considered statistically significant.

Mean (SE) UCP gelatinase concentrations differed among the collection methods, with the highest concentrations in SST tubes [192 (18) and 46 (4) µg/L for MMP-2 and -9, respectively] and the lowest concentrations obtained from 9NC tubes [102 (8) and 5 (1) µg/L for MMP-2 and -9, respectively]. MMP-2 concentrations appeared unaffected by anticoagulants, and MMP-2 was constitutively present in all UCB samples at 124 (11) µg/L.

Gelatin zymography of whole UCB revealed a constant band at
proMMP-2 at 72 000 and additional isoforms at M, 92 000, 130 000, and 225 000; these were biochemically recognized and immunologically identified as proMMP-2 and proMMP-9, respectively (Fig. 1A). UCB gelatinases are MMPs circulating as latent proenzymes in zymogenic activatable isoforms.

MMP patterns in UCP varied with different anticoagulants: samples collected in 9NC tubes showed mainly proMMP-2 at M, 72 000 [102 (8) μg/L] and little MMP-9 [5 (1) μg/L], whereas UCP from samples collected in dipotassium EDTA and lithium heparin, as well as serum, had additional proMMP-9 isoforms of M, 92 000, 130 000, and 225 000 [28 (3) μg/L; Fig. 1B]. MMP-9 isoforms were significantly higher in serum than in plasma [46 (4) vs 14 (2) μg/L, respectively; P < 0.01]; platelet aggregation during clotting may produce these differences (3). Anticoagulants added to the zymography buffer (concentrations equivalent to amounts in Vacutainer™ Tubes) almost completely inhibited the UCP gelatinases only in samples collected in dipotassium EDTA [residual activity, 4 (1)%; P < 0.01; Fig. 1A, lane 3], whereas other anticoagulants did not affect MMP activity (measured activity, 97–104% of the expected).

Cytometric analyses of UCB buffy coats collected with different anticoagulants revealed no differences in leukocyte subset recovery. Leukocyte subpopulations zymographically produced only MMP-9 isoforms [55 (7) μg/L; Fig. 1B, lanes 5 and 6]. UCB leukocytes thus apparently are the source of UCP MMP-9.

Umbilical cord vein extracts contained gelatinases mainly at M, 72 000 [71 (5) μg/L] and negligible MMP-9 activity, unaltered by anticoagulants except for dipotassium EDTA [residual activity, 3 (0.5)%]. Western blotting and biochemical analyses identified the gelatinase as latent activatable proMMP-2 (Fig. 1C).

We conclude that (a) anticoagulants alter UCB MMPs; unlike buffered sodium citrate, lithium heparin and dipotassium EDTA may induce the “release” of MMPs from UCB leukocytes, explaining the different UCP MMP profiles depending on the anticoagulant used (3); (b) umbilical cord leukocytes produce/secrete only MMP-9; (c) umbilical cord vein tissue is a source of the MMP-2 circulating in UCP; and (d) UCB MMPs may represent useful tools to identify pregnancy-associated syndromes (1, 2).

To avoid preanalytical misinterpretations and to optimize the diagnostic validity of UCB MMPs as biomarkers, we recommend the use of buffered sodium citrate, whereas use of serum or lithium-heparin and dipotassium-EDTA plasma, in which both MMP content and zymography are affected, should be avoided.

References
Plasma Concentrations of Cardiac Troponin I in Newborn Infants

To the Editor:

Ischemia and myocardial necrosis occur in 25–51% of newborn infants with perinatal asphyxia and are often associated with other adverse conditions specific to the neonatal period (1). Biochemical markers are more sensitive and specific than imaging techniques in the diagnosis of myocardial necrosis. Cardiac troponin I (cTnI) has high tissue specificity and sensitivity and is therefore suitable for use in diagnosing even microscopic lesions (2).

There is little information about cardiac biochemical markers in newborns, and no reference intervals have been established by the NCCLS standard procedures (3). The European Society of Cardiology and the American College of Cardiology suggest that cTnI and cardiac troponin T (cTnT) concentrations above the 99th percentile in a reference group be used as evidence of myocardial necrosis in adults (2). The present study was carried out to measure cTnI concentrations in plasma from healthy newborns to explore the possibility of a variation in reference values according to gender, age of the newborn, and plasma bilirubin and to suggest an upper limit for the reference interval, which is essential for the interpretation of these measurements in sick infants.

We consecutively enrolled 206 apparently healthy infants for whom bilirubin measurements had been requested because of diagnosis of jaundice. cTnI was measured in lithium-heparin-anticoagulated plasma samples on the ACS:180 immunoassay system, an automated system based on a two-sandwich immunoassay method and direct chemilumino-nometric measurement. The day-to-day imprecision (CV) for this assay, as performed in the core laboratory, for cTnI concentrations of 1.1, 16.1, and 34.1 µg/L was 8.3%, 9.4%, and 8.7%, respectively. Data from the manufacturer indicate a CV of 13% at 0.1 µg/L (4).

Data analysis was carried out with the SPSS program. The Student t-test was used to compare means. The reference interval was calculated from the percentiles of the empirical sampling distribution (nonparametric method), and the Dixon test (5) was used to identify outliers. The reproducibility of the method was estimated by calculating the mean and SD of the differences between 29 repeat measurements and their intraclass correlation coefficient. Statistical significance was established at P < 0.05.

The mean (SD) maternal age was 31 (5.1) years, and gestational age was 38.7 (1.2) weeks. One hundred and six infants (51.5%) were female. Patient selection adhered strictly to the study criteria: apparently healthy, with no problems that required surgical intervention, and no cardiac, neurologic, or other pathologies except jaundice. There were no adverse events associated with labor or delivery. The mean (SD) birth weight of newborn infants was 3200 (494.5) g, and the Apgar score was 9.1 (0.3) at 5 min. The mean (SD) age of the infants when blood was drawn was 2.6 (1.3) days. We identified no outlying values among any of the variables analyzed. The mean (SD) cTnI value was 0.28 (0.42) µg/L, the 99th percentile value was 2.8 µg/L, and the highest cTnI value was 3.0 µg/L (Table 1). The mean (SD) cTnI value for newborns younger than 48 h (n = 85) was significantly lower [0.22 (0.24) µg/L] than that for infants 48 h or older [n = 121; 0.37 (0.59) µg/L; P = 0.03]. We found no relationship between cTnI values and gestational age, plasma bilirubin, or gender. For 29 individual samples run in duplicate, the mean (SE) difference was 0.008 (0.132) µg/L (95% confidence interval, −0.28 to 0.26). The intraclass correlation coefficient for these same 29 repeat samples was 0.938 (SE = 0.07). The manufacturer does not supply reference values for

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