Cardiac Troponin Determination in Amniotic Fluid

To the Editor: Stefanović and Loukovaara have recently reported the increase in cardiac troponin T (cTnT) in amniotic fluid samples from perinatal period of 29 pathological pregnancies (1). Using amniotic erythropoietin (EPO) concentration as a marker of chronic fetal hypoxia, the authors found no detectable concentrations of either EPO or cTnT in amniotic samples from 5 uncomplicated pregnancies that served as the control group. In contrast, the found abnormal cTnT concentrations in 9 out of 29 pathological pregnancies, ranging from 0.01 μg/L (the detection limit of the assay) to 111.6 μg/L – a concentration rarely observed in adult myocardial infarction. The reported correlation between EPO and cTnT in all 29 pathological pregnancies was positive. However, we reanalyzed the data and observed a non-significant negative correlation between EPO and cTnT in these 9 cTnT positive abnormal pregnancies (Fig. 1, r= -0.23; 95% confidence interval -0.78 to 0.51), P=0.5457; data extracted from Table 1). Indeed, in case 3, where

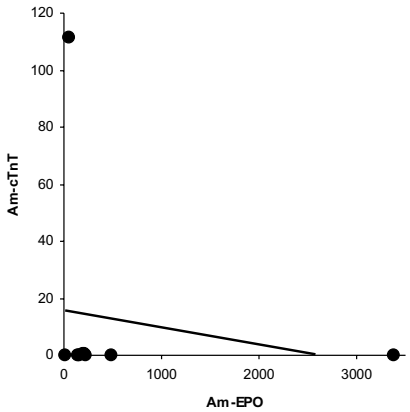

Figure 1. Correlation between amniotic erythropoietin (Am-EPO) and amniotic cardiac troponin T (Am-cTnT) in 9 cTnT-positive abnormal pregnancies (data reanalysis). r= -0.23 (95% CI, -0.78 to 0.51), *P*=0.5457.

the neonate was diagnosed with fetal hydrops, the reported EPO concentration (3,378 U/L) was the highest and yet the observed cTnT concentration (0.013 μg/L) one of the lowest. It is questionable if chronic fetal hypoxia as determined by EPO concentration reflects myocardial involvement. While there are differences in the developmental expression of cardiac troponin T and I (cTnI), it is currently unknown if cord blood cardiac troponin concentrations are of maternal or fetal origin (2). Further work including molecular studies is required to ascertain if the cardiac troponin is of diagnostic and prognostic use.

The validity of the analytical methods is also questionnable. Both the cTnT (Elecsys, Roche Diagnostics) and EPO (IMMULITE, DPC Ltd) immunoassays are intended for use in serum or plasma. Although the two technologies are different, the underlying principles used for detection are similar. Antigen-specific antibodies are employed to capture the antigen in the sample. This antigen-antibody complex binds a solid phase via a secondary detection antibody. A chemiluminescent substrate is added to the reaction, which gives out light when a voltage is passed across a magnetic spectrophotometer cell. The amount of light emitted is proportional to the concentration of the analyte. Although many improvements have been done in immunoassay design and sensitivity is enhanced by employing chemiluminescent detection system, all immunoassays have some pitfalls. In the case of cTnT (3), cTnI (4) and EPO, microparticle formation (normally lipoprotein aggregates) or fibrin microclots from incomplete clotting when obtaining serum or cellular debris may impede the washing steps in these assays. This results in the retention of antibodies within the measuring cell. This generates a signal possibly causing false-positive result. Immunoassays for cardiac troponin are known to yield false positive results due to fibrin microclot formation. As these matrix effects may also be true of amniotic fluid, caution in interpretation of cTnT concentrations is warranted.

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Author's Reply: David C. Gaze and Paul O. Collinson mainly cast doubt on the validity of the analytical methods and interpretation of the results in our study on use of cardiac troponin T (cTnT) in amniotic fluid samples and amniotic erythropoietin (Am-EPO) concentration as a marker of chronic fetal hypoxia.

We do not suggest that EPO directly regulates TnT or *vice versa*. Thus, it is not surprising to find a high concentration of Am-EPO in conjunction with low Am-TnT in individual cases. Importantly, the median concentration of EPO was 5-fold higher in pregnancies with high Am-TnT than in pregnancies with low Am-TnT. The difference between groups was only of a borderline significance ($P=0.051$), but the positive correlation between EPO and Am-TnT in the whole study population supports the idea that high Am-TnT may be a sign of fetal hypoxia. We agree that the validity of Am-TnT as a marker of fetal well-being needs to be further tested.

The validity of the analytical methods, especially immunoassay for determination of cTnT in the amniotic fluid is also questioned. We are aware that this assay is qualified only for use in the serum or in plasma. However, there are several reports on cTnT determination in different biological fluids (1,2). Ziebig et al (1) discussed the issue of validity of the analytical method for determination of cTnT and cTnI in urine. They were aware that troponin assays were not designed for the analysis of urine specimens, but in a control group (10 completely healthy individuals) all cTnI and cTnT measurements (plasma and urine) were below the detection limit. The within-assay imprecision in urine was14% and 3.3% (mean values of 0.016 and 0.263 μg/L, respectively) for cTnT and 4.8% and 3.2% (mean values of 0.647 and 8.42 μg/L, respectively) for cTnI. The assay was linear at urinary concentrations up to 0.01 μg/L for cTnT and 0.05 μg/L for cTnI. The recovery of troponin from urine, after the addition of defined quantities of plasma with cTnT and cTnI to urine, was 96-101% for cTnT and 98-103% for cTnI.

Tambar et al (2) found detectable concentrations of cTnT in the pericardial fluid of patient undergoing by-pass surgery. The Elecsys electrochemiluminescence immunoassay methods were used in both studies.

Gaze and Collinson also speculate that TnT in the amniotic fluid might be of maternal origin. Am-EPO as a marker of chronic fetal hypoxia has been routinely used in high-risk pregnancies in our hospital, and the validity of the assay has been tested (3).The molecular weight of EPO is 34 kDa, and EPO does not cross the placenta. As molecular weight of cTnT is almost the same, 33 kDa, one can expect the same inability for troponin to pass the fetoplacental barrier. Molecular weight of the lactic dehydrogenase is 35 kDa and of creatine kinase-MB 86 kDa. In our case 2, we observed extremely high concentrations of all markers of cardiac damage in the amniotic fluid while maternal markers were normal, which suggested the lack of transplacental permeability of myocardial injury markers. Adamcova et al (4) did not find the correlation between the concentration of cTnT in cord blood and maternal serum, nor did the other studies. They stressed out the significance of hemolysis on the cTnT detection. All our specimens were free of blood or thick meconium.

As we stated in the manuscript, the main purpose of the study was to investigate if cTnT appears in the amniotic fluid in a detectable concentration. As clinical significance of the detectable/increased concentration of cTnT and the turnover of cTnT are still unclear, our results did not influence the clinical decisions.

Further studies are required to determine the frequency of fetal myocardial injury, its severity and pathophysiology, and the implications for long-term follow up.

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