Placental corticotrophin-releasing hormone mRNA and microparticles in maternal plasma are not measures of placental shedding of debris: a rebuttal

M. BUIMER, C. A. R. LOK, R. NIEUWLAND, C. RIS-STALPERS and J. A. M. VAN DER POST
Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, the Netherlands

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The interesting article by Freeman *et al.* [1] reports an association between F-VII activity and corticotrophin-releasing hormone (CRH) mRNA but not number of circulating microparticles (MP) in pre-eclamptic patients. Their findings depend on the presumption that fetal CRH mRNA and number of MP are measures of placental shedding of debris. It is questionable whether this is the case.

The placental CRH system is a functional part of the fetal environment [2,3]. It is without doubt influenced by fetal stress and other factors associated with preterm delivery [4] and fetal growth restriction [5]. This makes placental CRH prone to confounding by fetal growth restriction and preterm delivery, which were different between the groups in the study by Freeman.

Furthermore, although a 1–6% fraction [6] of the MP population originates in the placenta, the majority (>95%) is derived from platelets [7]. As the prothrombinase assay measures the overall procoagulant activity of total MP, it is not specific for placenta-derived MP. To quantify placenta-derived MP, both flow cytometry and ELISA using NDOG2 or ED822 antibodies are more suitable [8]. Interpreting the concentration of circulating MP is further complicated by hemoconcentration and elevated platelet turnover in pre-eclampsia. Moreover, a fraction of the total MP population may be attached to maternal blood or endothelial cells.

In the presented study, total numbers of MP were comparable between groups. However, these data do not exclude involvement of specifically placenta-derived MP in the inflammatory response of pre-eclampsia because these MP were shown to trigger cytokine production by monocytes [9].

Finally, apart from quantification of MP, the presence of proteins on the MP membrane is of importance. Both positive and negative effects of MP have been reported that at least partially depend on the status of the parental cell. For example, MP from apoptotic T-cells impair relaxation, whereas MP from activated T-cells can increase NO release, thus promoting vasodilation [10].

Taken together, one can question if CRH mRNA and number of MP are appropriate markers of placental debris, which makes it hard to interpret the reported results and to use these measurements as indicators for placental dysfunction in pre-eclampsia.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Correspondence: Maarten Buimer, Department of Obstetrics and Gynaecology, Academic Medical Center, Amsterdam, the Netherlands.
Tel.: +20 5663454; fax: +20 6960441.
E-mail: m.buimer@amc.uva.nl

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Placental corticotrophin-releasing hormone RNA and microparticles in maternal plasma are not measures of placental shedding of debris: reply to a rebuttal

D. FREEMAN,* K. THAM,* E. A. BROWN,* A. RUMLEY,† G. LOWE† and I. GREER*
*Developmental Medicine; and †Division of Cardiovascular and Medical Sciences, University of Glasgow, Royal Infirmary, Glasgow, UK

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We agree with Buimer *et al*. [1] that the total number of microparticles in maternal plasma is not a measure of placental shedding of debris. We made our view clear in the discussion of our article [2] that 'the total microparticle population (in maternal plasma) comprises a mixture of fetal and maternal-derived microparticles and is not a specific marker of placental debris.' We did not claim that the overall procoagulant activity of total microparticles was specific for placenta-derived microparticles and stated 'our prothrombinase assay would detect total PS-exposing microparticles and would be unable to distinguish between fetal and maternal-derived material.'

While the letter of Buimer *et al*. [1] refers to our study as measuring the number or concentration of microparticles, this is not the case. Our interest in microparticle prothrombinase activity, and fetal CRH mRNA, in maternal plasma was in their interaction with the maternal coagulation system. We did not suggest that either measure would be a useful marker for placental dysfunction. Proteins are not the only molecules of interest with biological activity on the microparticle membrane. In the process of apoptosis, phosphatidyl serine molecules, which normally reside in the inner leaflet of the cell membrane, flip over to the outer leaflet. This alerted us to the possibility that the exposed PS could act as a platform for assembly for coagulation factors. Rather than using FACS to identify the number of microparticles, we used the prothrombinase assay, which is dependent on the expression of PS molecules on the surface of the microparticles; hence our reference in the article to 'phosphatidylserine-exposing microparticles' and our unit of measurement of nM PS equivalents [2]. Because we are not measuring actual numbers of microparticles but relating their biological activity to measures of coagulation activation in the same sample, hemoconcentration is not a concern. These phosphatidylserine-exposing microparticles may represent yet another subset of the total microparticle population but one that can be identified by its biological activity.

We do state that we consider fetal CRH mRNA levels in maternal blood to be one measure of placental debris. CRH is made by cytotrophoblasts. Placental CRH mRNA is subject to upregulation in situations of fetal stress and placental dysfunction. There appears to be little doubt that fetal CRH mRNA expression is upregulated in pre-eclampsia, as indicated by a number of cDNA array experiments; see, for example, Nishizawa *et al*. [3]. Therefore, as Buimer *et al*. point out, we cannot discount the possibility that our observation of increased fetal CRH mRNA levels may result from no change in shedding of placental debris but that each 'package' of debris contains more copies of CRH transcript. This could potentially confound our observed relationship between fetal CRH mRNA and factor VIIa activity. However, if fetal CRH mRNA was merely a non-specific marker for the presence of a pre-eclamptic placenta, we might have expected to observe a

Correspondence: Dilys Freeman, Developmental Medicine, University of Glasgow, Royal Infirmary, University Block, 10 Alexandra Parade, Glasgow, UK.
Tel.: +44 141 211 4706; fax: +44 141 552 0873.
E-mail: d.freeman@clinmed.gla.ac.uk

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