Reviewer's report

Title: Elevated PDGF-BB Concentrations in Premature Neonates Who Develop Chronic Lung Disease

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Reviewer: Willem A Dik

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Review
Elevated PDGF-BB concentrations in premature neonates who develop chronic lung disease (Adcock et al)

Neonatal respiratory distress syndrome (RDS) may progress towards the chronic lung disorder called chronic lung disease of prematurity (CLD) also often referred to as bronchopulmonary dysplasia (BPD). CLD is characterized by disturbed lung development, reflected by decreased alveolization and varying degrees of fibrosis. However, sparse data are available on the role that fibrogenic growth factors may play in CLD development.

PDGF-BB is a growth factor for fibroblasts and a role for this growth factor is suggested when pulmonary fibrosis develops. This study examines the presence of PDGF-BB in tracheal aspirates (TA) in infants who are mechanically ventilated because of RDS and tries to correlate PDGF-BB levels in TA with CLD development as well as pulmonary hemorrhage (PH). Although the link between PDGF and fibrotic lung disorders is nothing new, this study is potentially interesting for people examining the pathogenesis of RDS and CLD. However, there are some major concerns that should be addressed by the authors.

Major compulsory revisions:

1). In the materials and methods section it is mentioned that, after collection, TA's were centrifuged at 14,000 g for 10 minutes. This methodology might introduce a very important problem resulting in meaningless PDGF levels in TA and in such makes the whole study irrelevant. From the materials and methods section it appears that inflammatory cells (which are present in high numbers in TA of babies suffering from RDS/CLD) were still present in TA at the time of centrifugation (14,000 g for 10 minutes). Inflammatory cells are well known to contribute to increased PDGF levels in various fibrotic lung diseases. By centrifugation of the TA's (14,000 g) that still contain inflammatory cells PDGF might have been artificially released from the cells into the TA fluid as cells will collapse when spun down with such a force. If this is the case this influenced the PDGF levels in the TA's making the PDGF measurements unsuitable for drawing conclusions. The authors should comment on this topic.

2). There are infants included in this study that suffer from PH. It is not surprising at all that PH itself is associated with increased PDGF-BB levels in TA. However, at what time was PH diagnosed in these infants? In other words do the infants with PH cause the increase of PDGF-BB levels in the TA's at days 4 and 6 as depicted in figure 1? The authors should state when PH was diagnosed and whether PH biased the result to increased PDGF-BB levels between at days 4 to 6 as presented in figure 1. If the patients with PH did bias the measurements at days 4 to 6 to increased PDGF-BB levels it is impossible to conclude that PDGF peaked between days 4 and 6 of life as most patients did not suffer from PH.

3). The authors state that TA PDGF-BB concentrations correlate with the development of BPD/CLD. However, the infants with PH are more likely to develop BPD/CLD. Again, do these infants account
for the fact that in figure 2 A and B the levels of PDGF-BB are higher in the infants developing chronic lung disease compared to the ones recovering from RDS. This should be addressed otherwise the conclusion drawn from these data are not valid in my point of view. In addition to this the differences in PDGF between resolving disease and progression toward chronic lung disease are not significant.

4). From figures 1 and 2 it is unclear how many TA were analysed per time point (I assume that only one sample per patient was examined per time point, is this the case?). The data in figures 1 and 2 should be replaced by dot blots depicting every TA sample (patient) per time point as a dot. In this figures median values should be given instead of mean values. Presenting data such way provides insight in the number of subjects represented by each time points as well as the variation of PDGF levels between the different patients per time point (this variation appears to be considerable regarding that SEM values are given in figures 1 and 2 from the current draft of the manuscript).

5). The authors state that because of the fact that infants varied with respect to the timing of their inflammatory process the relationship of maximal PDGF-BB concentrations and the development of chronic lung disease was examined. How did the authors determine the timing of the inflammatory process? Cell counts and differentials or cytokine measurements should be provided then. Are max PDGF levels in most infants developing chronic lung disease detected at days 4 and 6?, which is suggested by figure 2.

6). In the discussion section the authors state that peak concentrations of PDGF occur within the first week of life when CLD developed. Then they mention that this is consistent with a study by Currie et al demonstrating mitogenic activity in BAL fluids from infants developing CLD and those resolving from RDS. However the study by Currie et al shows no difference in mitogenic activity of BAL fluid from RDS or CLD during the first week of life. Furthermore, Currie and colleagues were unable to demonstrate a role for PDGF-BB in this mitogenic activity. In my opinion the fact that Currie and colleagues were unable to block PDGF activity in BAL fluid was due to the fact that they used antibodies to block PDGF activity. We also performed these kind of studies and were also unable to block PDGF activity in BAL fluid by using antibodies. In a recent manuscript from our lab (Eur Respir J 2003; 21: 842-847) it was demonstrated that, using an alternative way of blocking PDGF activity, PDGF does contribute to the mitogenic activity of BAL fluid. Therefore, if the authors want to suggest a possible role for PDGF in stimulating fibroproliferation during CLD this reference should be added.

7). The authors should state whether steroid treatment did influence PDGF levels.

Minor compulsory revisions:

1). From which company was the recombinant PDGF used to generate the standard curve for the ELISA procedure?

2). The authors describe that very low birth weight infants were used in their study. However they include infants with a weight of less then 1500 grams while real low birth weight infants are considered to have a weight of less than 1000 grams.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No

Declaration of competing interests:

none