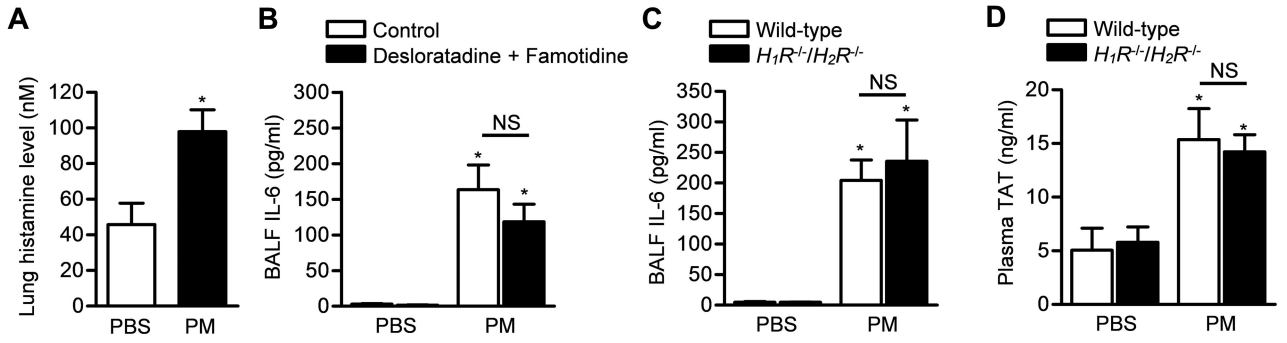

Figure 1. The effect of loss of histamine signaling in particulate matter–induced thrombin generation. Fine urban particulate matter (National Institute of Standards and Technology Standard Reference Material, SRM 1649a 200 μg/mouse in 50 μL PBS) or vehicle was administered intratracheally to 20-25 g, 6-8 week old male C57BL/6 mice as previously described.[4] (A) After 24 hours BAL fluid was obtained and histamine levels were measured using a commercially available assay (EIA Histamine IM2015, Beckman Coulter). (B) Mice were treated with famotidine (10 mg/kg) and desloratadine (10 mg/kg) in 150 μL of PBS 4 hours before treatment with PM followed by an additional dose of famotidine 8 hours later. Twenty-four hours after PM administration, BAL fluid was obtained and IL-6 levels were measured as previously described (ELISA).[3] (C,D) H1R and H2R receptor double knockout mice (*H1R*$^{-/-}$/*H2R*$^{-/-}$) or littermate controls were treated with PM and 24 hours later IL-6 and TAT were measured in BAL fluid and citrated plasma as previously described.[3] The protocol for the use of mice was approved by the Animal Care and Use Committee at Northwestern University. N = 4 or 5 animals for each group. *$P < .05$ compared with PBS control; and NS, not significant using ANOVA with Bonferroni posttest comparison.

well-described in the hematology literature.[6] The potential clinical importance of this mechanism is highlighted by the frequent observation in human populations exposed to particulate matter that IL-6 or its transcriptional target, C-reactive protein, are increased the day after the exposure.[7-9]

Like Nemmar and colleagues, we reported that the administration of particles resulted in an influx of macrophages and neutrophils into the lung.[3] However, we found that, while the instillation of PM increased histamine levels in the bronchoalveolar lavage (BAL) fluid (Figure 1A), the PM-induced increase in bronchoalveolar lavage fluid IL-6 was not affected by combined pretreatment of mice with pharmacologic inhibitors of histamine type I (H1R) and type 2 (H2R) receptors (Figure 1B). Furthermore, the PM-induced increase in BAL fluid IL-6 and the subsequent increase in plasma TAT complexes were similar in mice doubly deficient in the H1 and H2 receptors and their littermate controls (Figure 1C-D). As Franchini and Mannucci point out, much more work is required before we understand the important link between particulate matter air pollution exposure and thrombosis.

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To the editor:

Prediction of fetal status in fetal/neonatal alloimmune thrombocytopenia (FNAIT)?

We read with interest the recent paper from Bertrand et al,[1] which outlines an interesting topic regarding the use of antibody concentration as predictive parameter for FNAIT.

Unfortunately, the number of subjects studied is very low. When the authors conclude that FNAIT is a severe disease and the first offspring is already at high risk, they base their analysis on 66 "index cases." A significant difference in antibody concentration between primagravidae and multigravidae, however, is concluded from the analysis of 54% (20/37) and 34% (10/29) of enrolled cases only. Selection criteria are not given. In consequence, complete

data were only available from 30 subjects over a period of 27 years. As the incidence of FNAIT is 1:1000 live births, and 600 FNAIT cases can be expected in France per year, we fear that the number of subjects may be too small to draw general conclusions.

More specifically, the following aspects require special attention:

We believe that the design of the study does not allow concluding that no significant correlation exists between the HLA-DRB3 allele and the maternal alloantibody concentration at delivery. Primary data are missing. However, the frequency of this allele among mothers of children with anti–HPA-1a–mediated FNAIT is as high as 98%.[2] Accordingly, a significant difference in the frequency of this allele between any selected groups of mothers is practically excluded a priori and should always prove statistically insignificant.

We also believe that the statistical methods applied to conclude that the most efficacious treatment is maternal therapy with IVIG and steroids are insufficient. The conclusion is drawn from 155 treated pregnancies (not 239 pregnancies as stated in the "Patient cohort" section). Statistical comparisons between platelet counts of newborns assigned to different treatment groups were made with the Mann-Whitney U test, which requires independent samples. This requirement is violated by the fact that the study compares unrelated newborns plus (groups of) siblings born to the same mothers. In addition, the "no treatment" group consists of index cases, that is, (almost exclusively) of first-born babies. This bias may have a systematic influence on platelet counts independent from therapy.

Finally, the authors state that the maternal alloantibody concentration during pregnancy is predictive of fetal thrombocytopenia. Readers should note that the negative predictive value in this study was 75%; accordingly, every fourth unborn will go untreated although it does require treatment. The authors state that their recent study confirms their own data from a previous report.[3] Such a cross-reference is of value if the 2 study groups are independent. We noticed that in the former study, 27 cases were enrolled between 1984 and 2004; and in the recent study, 28 cases were collected from 1981 to 2009. Furthermore, the threshold of the antibody concentration was lowered by a factor of 10 and the scientific and statistical justification for the definition of these different cut-off values is missing.

Taken together, several limitations apply when interpreting this study. Data are not yet sufficient to allow for guiding the management of pregnancies in FNAIT and we should still consider whether antibody concentration will prove helpful in future an open question.

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To the editor:

The pathophysiology of FNAIT cannot be deduced from highly selected retrospective data

Today there is no consensus regarding the optimal method for determining the fetal status when the mother has alloantibodies against fetal platelets. Therefore, it was with great interest we read the article by Bertrand et al[1] recently published in *Blood*. However, we feel that a few points need to be clarified by the authors.

The study[1] included 75 women who underwent 239 pregnancies complicated by fetal and neonatal alloimmune thrombocytopenia (FNAIT). Given the same frequency of FNAIT in France as in Norway,[2] the yearly expected number of newborns with FNAIT in France would be between 400 and 700. It is surprising that the authors report only the result of 155 pregnancies in Table 2. This represents 0.8%-1.4% of the total number of FNAIT cases occurring in France[3] and 4.7%-8.5% of all FNAIT cases in Paris.[4] Furthermore, of the 75 women who were studied, we were puzzled to discover the authors present antibody values at delivery from only 30 of these women. The very low number of pregnancies included clearly indicates that the cohort of patients is not a representative selection of FNAIT cases, and in our view it is very unlikely that the authors' findings can be generalized.

Ensuring homogeneity between groups is very challenging when data collection has taken place over 3 decades. The authors state that "… homogeneity of the subgroups was carefully controlled (cases collected between 1981 and 2009)," [1p3209] but they do not explain how they controlled for homogeneity.

Without giving any data, the authors state that "no significant difference emerged between the concentrations of the untreated mothers of blood groups A and O" and they "did not observe any significant correlation between HLA-DRB3 allele and the maternal alloantibody concentration at delivery or the neonatal platelet counts." [1p3210] The reader cannot evaluate whether the lack of significance is because of a type II statistical error, and it is therefore impossible to assess the validity of the authors' claims.

To predict severe thrombocytopenia from maternal alloantibody levels the authors have calculated sensitivity, specificity, and positive and negative predictive value (see Bertrand Table 4[1]). The