ROUTINE FETAL PLATELET GENOTYPING BY ddPCR : OVERVIEW OF THE FRENCH EXPERIENCE

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Background

Fetal platelet genotyping is essential for the diagnosis of fetal-maternal platelet incompatibilities and for the management of fetal and neonatal alloimmunization thrombocytopenia (FNAIT). In Caucasians, the majority of FNAIT is due to the 4 human platelet antigen (HPA) systems HPA-1,-3,-5, and -15. Noninvasive prenatal testing (NIPT) is based on the detection of fetal DNA in maternal plasma using different methods: PCR techniques or next generation sequencing or digital PCR.
Our laboratory has developed a new tool based on droplet digital PCR (ddPCR) for fetal platelet genotyping in maternal plasma. Based on the article by Milbury et al, *Biomol Detect Quantif*, 2014, a long period of validation was necessary to define the critical parameters of the ddPCR technology in order to interpret the results. This work resulted in the publication of an article in 2020 by Ouzegdouh Mammasse Y et al, *Br J Haematol*. Since October 2020, fetal platelet genotyping is part of the analytical offer of the laboratory.

Aim

The aim of this study was to confirm or not the results obtained previously concerning the representation of HPA systems involved in fetomaternal incompatibilities.

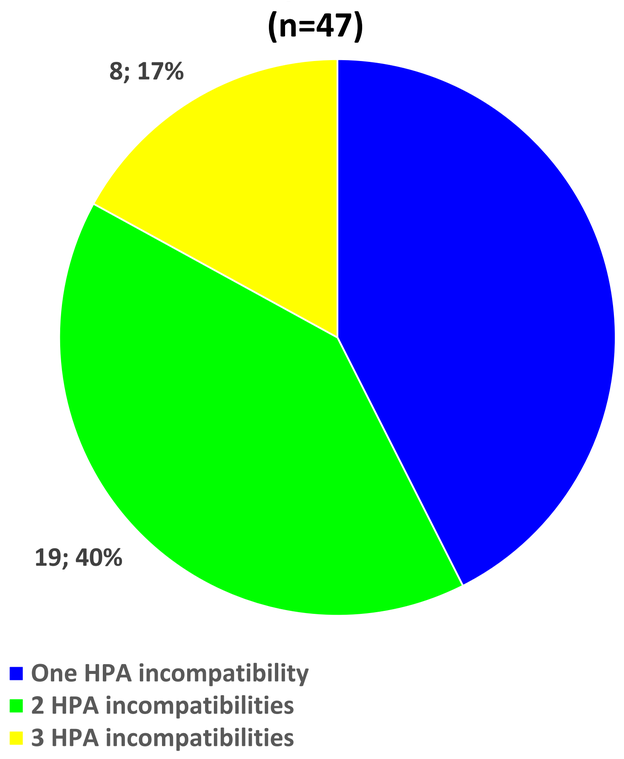
Methods

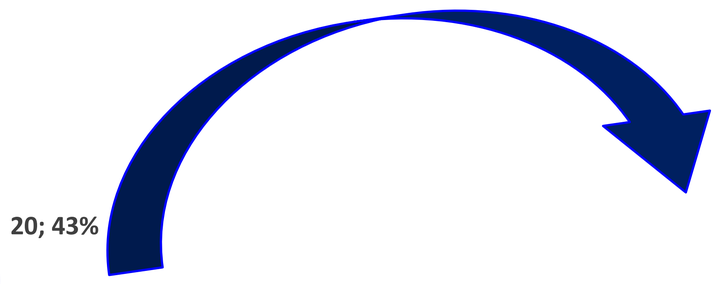
In this study, two samples of 20 mL of blood on Cell Free DNA tube were collected at 15 days interval or less according to the term of pregnancy. After extraction of circulating fetal DNA (QIAamp Circulating Nucleic Acid, Qiagen, Germany), fetal platelet genotyping was performed on the QX200 Droplet PCR System (BioRad).

Results

47 pregnant women were tested according to international recommendations: heterozygous father/homozygous mother (n=43) or absent father (n=4) in the context of a history of FNAIT or intracranial hemorrhage (ICH) or discovery of ICH during pregnancy, 26 patients were immunized by alloantibodies, mainly anti-HPA-5b (n= 21 or 44.7%). The term of the first determination varied between 12 WG and 33 WG + 3D.
47 patients had a potential incompatibility with their spouse in: 1 single HPA system in 42.5% of cases (n=20), 2 systems in 40.4% of cases (n=19) and 3 systems in 17% of cases (n=8) (Figure 1).
32% of the fetuses were compatible with their mothers whereas 53% were incompatible in 1 system, 13% in 2 systems and 2% in 3 systems (Figure 2). The HPA-1 system alone accounted for 10.6% of incompatibilities, 19% in combination with other systems. On the other hand, the HPA-5 and HPA-15 systems alone represent 25.5%, 58% in association with other systems.

Figure 1 : Potential HPA incompatibilities between both spouses (n=47)

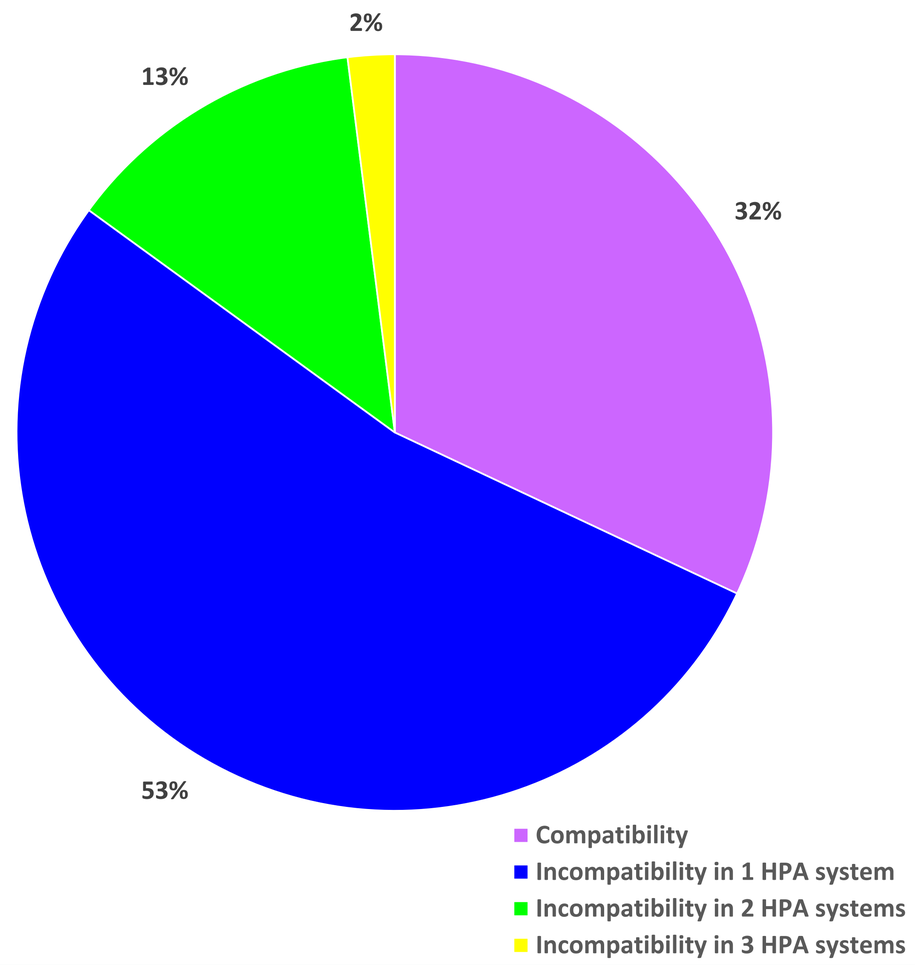




HPA-1
alone : 10.6%
in association with other HPA systems : 19%

HPA-5 and HPA-15
alone : 17% and 8.5% respectively
in association with other HPA systems : 58%

Figure 2 : Fetal platelet genotyping status versus mother genotyping (n=47)



Conclusion

This study shows that the recruitment of the laboratory differs from the literature, the patients present several incompatibilities with their spouse and are mainly immunized by an anti-HPA-5b allo-antibody. These results support the interest of having developed fetal platelet genotyping in 4 HPA systems simultaneously. This work should be pursued prospectively in order to improve practices and management recommendations, as many patients are at a very advanced stage of pregnancy. .