

CLINICAL INPUT OF ANTI-D QUANTITATION BY CONTINUOUS FLOW ANALYSIS ON AUTOANALYSER IN THE MANAGEMENT OF SEVERE ANTI-D MATERNAL ALLO-IMMUNIZATION

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BACKGROUND Beside titration by indirect antiglobulin test most widely used, anti-D quantitation by continuous flow analysis (CFA) may be performed to assess maternal immunization severity. Only few studies have related its interest in the management of pregnancies complicated by anti-D immunization. To reduce the severity of the hemolytic disease of the fetus and the newborn (HDFN) in pregnancies at higher risk of early intrauterine fetal transfusion (IUT), a preventive high-dose intravenous immunoglobulin (IVIG) therapy may be introduced. The effect of IVIG treatment on maternal anti-D CFA concentrations has not yet been established.

AIMS The aim of our study was firstly to demonstrate the relevance of anti-D CFA quantitation for the management of pregnancies complicated by anti-D immunization and secondarily to assess its added value for the biological follow-up of patients treated with IVIG treatment.

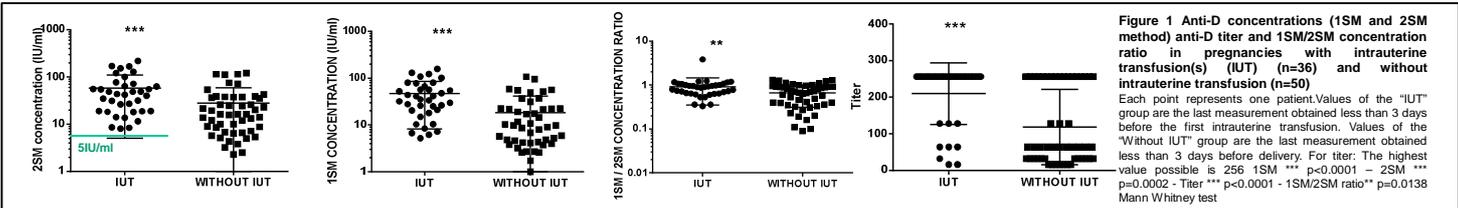
METHODS A retrospective study of 86 severe anti-D immunized pregnancies (anti-D titer > ou = 16) followed at the Trousseau hospital between 2013 and 2014 was conducted. Concentrations of maternal anti-D were measured by CFA 2-stages method (2SM) (total amount of anti-D) and 1-stage method (1SM) (high affinity IgG1 anti-D). Simultaneously, titrations were performed. These biological datas were compared to the severity of the antenatal HDFN (need of IUT, gestational age of the first IUT, fetal cord hemoglobin concentration before the first IUT). For 6 severely anti-D allo-immunized pregnant women treated with IVIG and followed at the Trousseau Hospital between 2013 and 2015, the kinetic of anti-D CFA concentrations was analyzed since IVIG introduction and until the first IUT.

RESULTS The value of 5IU of anti-D /mL in maternal sera is validated as a threshold to trigger ultrasonographical and doppler fetal surveillance in order to detect fetal anemia. For pregnancies requiring IUT (n=36), maternal 1SM anti-D concentration correlates significantly with the severity and the precocity of the fetal anemia. In 5/6 pregnancies with IVIG treatment, anti-D 1SM and 2 SM concentrations significantly decrease after IVIG introduction, suggesting that one of the mechanism of action of this treatment to delay the outcome of the fetal anemia is the reduction of the maternal antibody load.

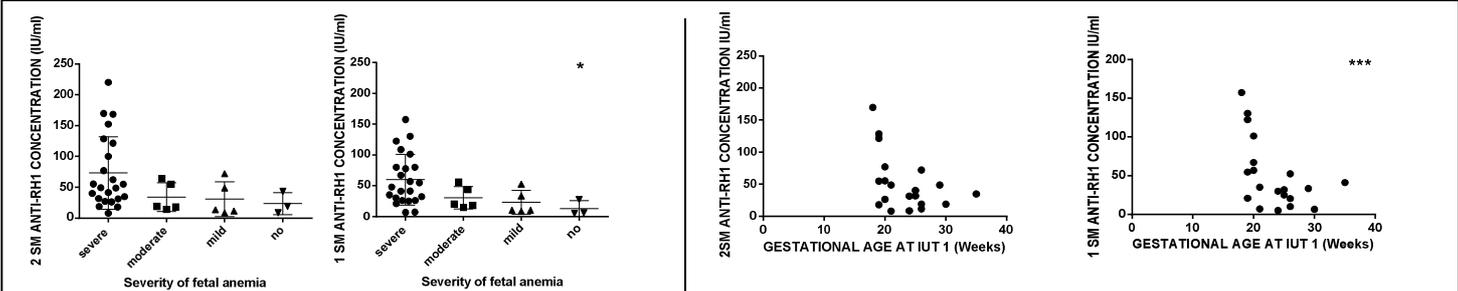
CONCLUSION Altogether our results underline the interest of anti-D quantitation by CFA to optimize the management of severe anti-D allo-immunized pregnancies.

Follow-up of pregnancies complicated by severe anti-D allo-immunisation

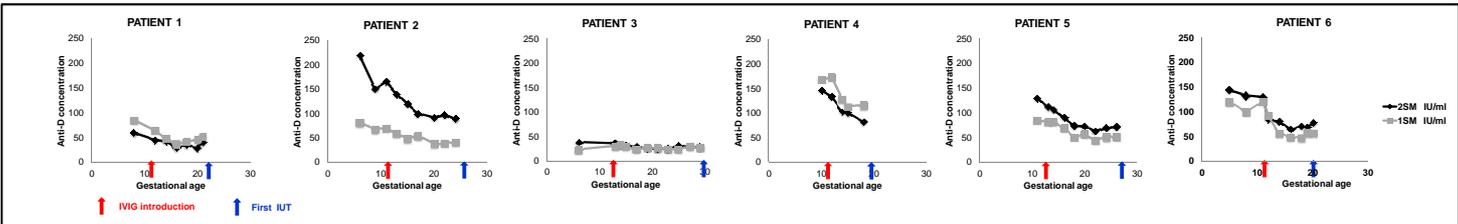
 <p>titration</p>  <p>2SM CFA: measure of the agglutination of prebromelinated D positive red blood cells induced in the continuous flow by the anti-D antibodies present in the serum of the patients</p> <p>1SM CFA: bromelin is added inside the continuous flow : IgG3 are destroyed and only high affinity IgG1 bind and agglutinate native D positive red blood cells.</p>	<p>Biological follow-up:</p> <p>Anti-D quantitation every 2 weeks since 18 weeks of gestation (WG) with titration, 1-stage method (1SM) and 2-stages method (2SM) continuous flow analysis (CFA)</p>	 <p>Ultrasonographical surveillance:</p> <p>Fetal red blood cells hemolysis due to maternal anti-D antibodies could lead to severe fetal anemia, hydrops fetalis and fetal death. In cases of severe maternal allo-immunization, fetuses are monitored every week since 16WG by ultrasonography. Fetal anemia could be diagnosed in a non invasive way by measurement of the peak systolic velocity in the middle cerebral artery (MCA-PSV)</p>	<p>Intrauterine fetal transfusion (IUT)</p> <p>If severe fetal anemia is diagnosed, fetal red blood cells transfusion could be performed to avoid complications.</p> 
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Patients in the « IUT » group have higher anti-D titer, 1SM and 2SM concentrations and 1SM/2SM ratio. The presence of an overlap of anti-D concentrations in the 2 groups shows that anti-D CFA could only be used as screening tests to determine when clinicians should monitor the pregnancy with MCA-PSV measurement. 5 IU/ml appears to be a good threshold. Others factors that have to be determined may condition the fetal outcome (in particular factors which influence the maternal antibody response, the placental transfer of maternal antibodies and the destruction of fetal red cells)



In the IUT group, 1SM anti-D concentration correlates with the severity and the precocity of the HDFN. These results underline the pathological role of IgG1 anti-D antibodies during the pregnancy. These antibodies are known to cross the earliest the placental barrier (since 13 GW).



IVIG treatment induces a decrease in antibody concentrations (1SM and 2 SM) in 5/6 cases (except patient 3). It also appears to delay the outcome of a fetal anemia in at least 2/6 cases (patients 2 and 5) when comparing the initial antibody concentration and the gestational age of the first IUT with those reported in the reference population (see figure 3).

Anti-D CFA quantitation appears to be one of the most relevant methods to manage pregnancies complicated by severe anti-D alloimmunization and is the method chosen in the 2 specialized labs in France. Antibody dependent cellular cytotoxicity tests (NK cells or monocytes) or mononuclear phagocyte assays are also a relevant but a more complex alternative and they are performed only in a few countries. Better tests that will take into account both fetal and maternal specific factors need to be developed. IVIG treatment is performed only in pregnancies at higher risk of early IUT. It induces a diminution of the antibody load in most cases and our results suggest that, in certain cases, it may delay the outcome of the fetal anemia. The mechanism of action of this treatment remains to be established (blockade of FcRn or of activating FcγR, upregulation of the inhibitory FcγRIIb, neutralization of anti-D antibodies by anti-idiotypic antibodies...). Alternative and more efficient immunomodulatory treatment need also to be developed but the difficulty of conducting clinical trials in pregnant women and the absence of relevant murine models make matters difficult.