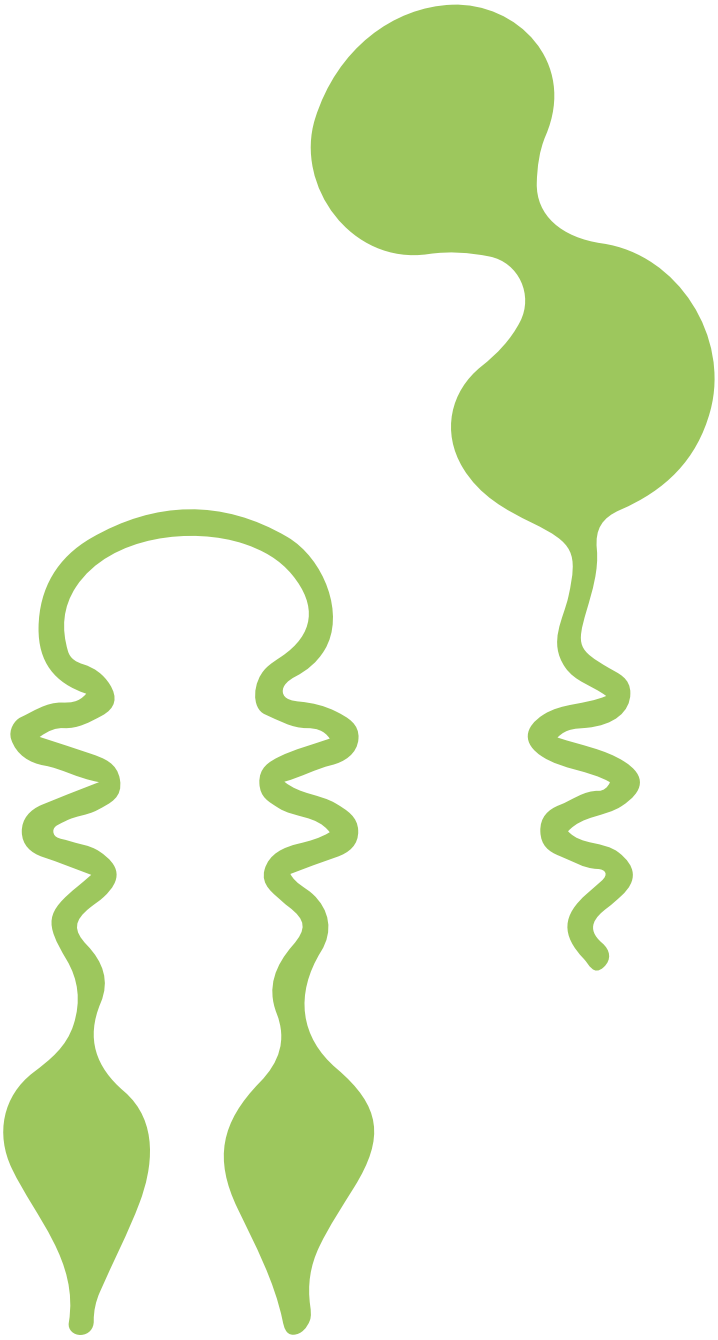
The diagnostic value of plasma thrombopoietin levels and platelet autoantibodies

Porcelijn, L.



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CHAPTER 6

Lack of detectable platelet autoantibodies is correlated with nonresponsiveness to rituximab treatment in ITP patients

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Lack of detectable platelet autoantibodies is correlated with nonresponsiveness to rituximab treatment in ITP patients

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A complete list of the members of the Dutch HOVON 64 Study Group appears in the "Appendix."

Rituximab, a chimeric CD20 monoclonal antibody that causes depletion of B cells, shows a short term treatment efficacy in 40% to 60% of immune thrombocytopenia (ITP) patients.1,2 Our recently published multi-center randomized open label phase 2 trial comparing three rituximab dosing schemes showed 50% responses in 138 ITP patients.3 Unfortunately, the treatment efficacy of rituximab so far cannot be predicted. Here, however, we show absent levels of platelet-bound antibodies to be associated with refractoriness to rituximab. Assays on platelet bound antibodies may hence lead to a more personalized treatment approach for these patients.

ITP was diagnosed in accordance with the recommendation of the American Society of Hematology (ASH)4, by patient and family history, physical examination and laboratory investigations (e.g. normal WBC count and differentiation, normal RBC count, RBC indices and mean platelet volume, absence of HIV, HBV,HCV, Helicobacter Pylori, and antinuclear factor, antinuclear antibodies, antiphospholipid antibodies). Furthermore, low to normal thrombopoietin (Tpo) levels supported thrombocytopenia to be due to increased platelet destruction.5 Eligible patients were 18 years of age or older, with an ITP relapse or refractoriness (minimally two platelet counts < 30 x 109/L), at least 3 weeks after high-dose (≥ 1 mg/kg) corticosteroids and who were randomized between three rituximab dosing schemes, i.e. 2 or 4 once-weekly standard 375 mg/m2 doses and a twice-weekly 750 mg/m2 regimen.3 Complete (CR) good/partial (PR) and moderate (MR) response were defined as platelet counts of 150x109/L or more, 50x109/L

or more on 2 consecutive occasions and a platelet count over 30x109/L with at least twice the base-line count, respectively. The different dosages of rituximab did not lead to changes in response rate.3

Prior to starting the first rituximab dose and subsequently for ten weeks, weekly EDTA-anticoagulated blood sampling was requested to assess the presence of platelet-associated IgG and IgM autoantibodies by the direct platelet immunofluorescence test (PIFT) and the eluate-(indirect) PIFT, as described previously.6 In the current report, a positive 'PIFT' refers to the combination of a reactive (=1+ to 3+) direct as well as eluate PIFT. One or both tests with negative reactions were defined as a negative 'PIFT'. Additionally, in samples with sufficient platelet numbers, both the PIFT and a direct 'monoclonal antibody immobilization of platelet antigens' (MAIPA) assay were performed; the latter to detect IgG-class autoantibodies.7 All MoAbs, CLBthromb/1 (CD41, anti-GPIIb), MB45 (CD42a, anti-GPIX) and SW16 (CD42d, anti-GPV) were supplied by Sanquin Reagents (Amsterdam, the Netherlands).

For statistical analysis: correlation between continuous variables was calculated by Spearman's rank test, association between categorical valuables was calculated by the 2-tailed Fisher Exact probability test and 95% confidence intervals were calculated using Stata version 14.0 (StataCorp LP, Texas, USA).
Prerituximab treatment samples from 112 of 138 patients and follow-up samples during and after rituximab treatment from 80 patients were sent to our laboratory (Supplemental Figure 1).

In 99 of 112 patients samples (88%), the platelet count in prerituximab treatment samples was sufficient to enable platelet associated auto-antibody detection by PIFT. Of these, a representative 47 (47%) responded to rituximab (CR: n=17, PR: n=22 and MR: n=8, respectively) while 52 (53%) were nonresponders. For all tested patients (n=99) direct PIFT results corresponded with the eluate-PIFT results. Antibodies were present in 79 patients, of whom 43 resonded (54%) with 16 complete (21%). In contrast, the absence of antibodies in 20 patients was associated with 4 responses (20%) of which only 1 (5%) was complete (Table 1, p=0.006). In summary, undetectable platelet autoantibodies in the direct PIFT strongly predict absence of or less than complete response to rituximab (Table 1: negative predictive value 95.0% ;CI 73.2-99.25%, positive predictive value 20.3%; CI 17.7-23.1%).

Vice versa, 16 patients (94%) with a complete response to rituximab showed positive to very-strong-positive direct PIFT reactions (mean level 2+) whereas the percentage of non-responsive patients with a positive direct PIFT was much lower (69%; p=0.051). In patients with partial or moderate platelet responses, positive

PIFT occurred with similar frequency in 19 (86%) and 8 (100%) respectively.
Serial autoantibody testing was performed for 80 of the sampled patients (71%). Of these, the presence of platelet autoantibodies and corresponding platelet counts were evaluated “per response group” at baseline and at the time of the highest platelet counts that were reached within 10 weeks after start of rituximab treatment (Figure 1; supplemental Figure 2). Of 30 patients predominantly responding (10 CR, 10 PR, 3 MR), the platelet count also enabled the direct MAIPA. Both PIFT and MAIPA results in these patients appeared in full concordance, i.e. seven patients (4 NR and 3 PR) were negative with both PIFT and direct MAIPA and 23 (3 NR, 3 MR, 7 PR and 10 CR) positive with both PIFT and direct MAIPA.

CR in the Haemato Oncology Foundation for Adults in The Netherlands (HOVON) 64 Study was defined as a rise in platelet counts to 150x109/L or more. Nowadays, ITP is defined as platelet counts <100 x 109/L.8 Re-categorization of the response groups, using 100 x 109/L did not result in different response numbers.

	detectable platelet-associated antibodies at baseline		
	Positive (%)	Negative (%)	total
CR	16 (20)	1 (5)	17
PR	19 (24)	3 (15)	22
MR	8 (10)	0 (0)	8
NR	36 (46)	16 (80)	52
total	79	20	99

P=0.006

Anti-CD20 monoclonal antibodies deplete B cells for periods varying from months to more than one year. In this respect B cells are essential to present antigens to CD4+ T cells and moreover secrete cytokines activating macrophages, dendritic cells and immune-regulatory cells and thus an ongoing autoimmune response.9-11 Additionally, rituximab is reported to normalize both the abnormalities and dysfunction of the T cell compartment in ITP patients.9,12-14 However, depletion of pre-plasma cell B cells and with it decreased autoantibody production is regarded as most likely mechanism of action. But, so far not one of these explanations could be correlated to rituximab’s variable therapeutic effects.

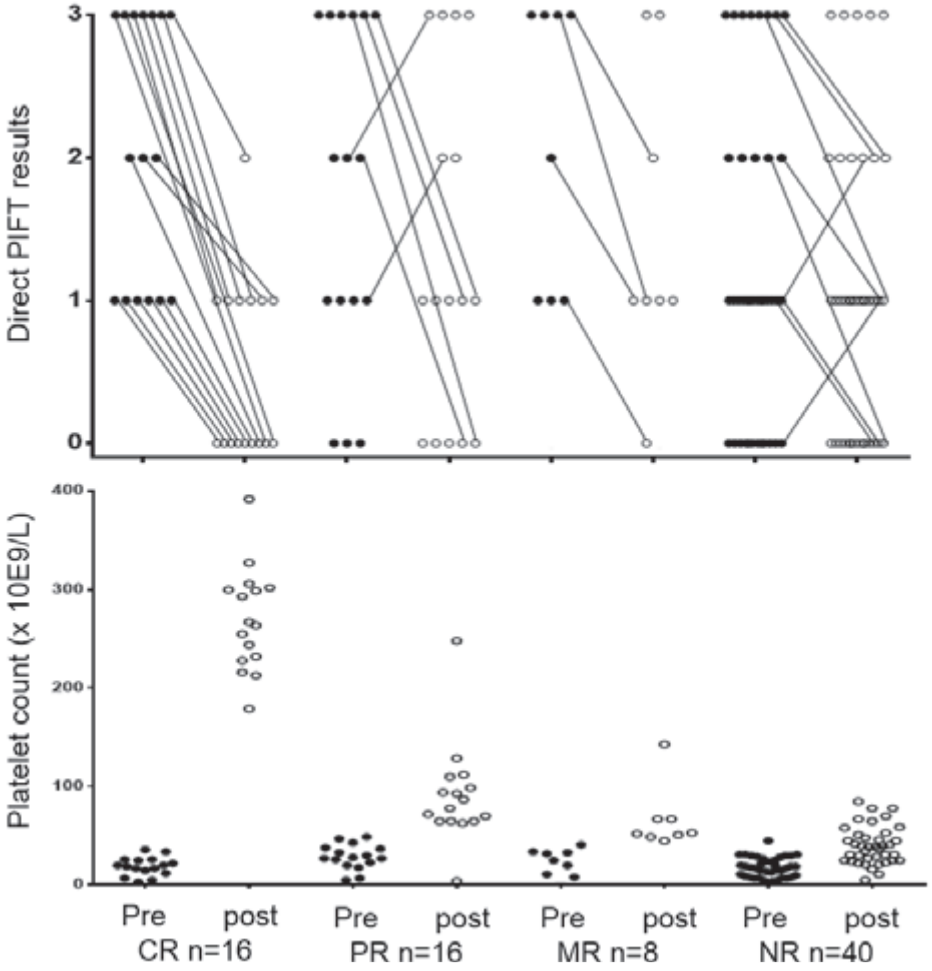

***Figure 1**. Direct PIFT results and platelet counts prior to initiation with rituximab and at the time of the highest platelet counts reached within 10 weeks after start of rituximab treatment for 80 serially tested ITP patients. X-axis for figure B also applies for figure A. A: CR=complete responders (n=16), PR=partial responders (n=16), MR=moderate responders (n=8), NR=non-responders (n=40). Direct PIFT scores before (pre) and at the time of the highest platelet count within ten weeks after (post) treatment with rituximab: 0=negative, 1=positive, 2=strong positive and 3=very strong positive. Changes in antibody detection results are indicated by means of connecting lines.*

B: Platelet counts before and the highest platelet count within ten weeks after treatment with rituximab.

Our results are in agreement with the latter mechanism while all serially tested CR patients (n=16) showed decreasing antibody results and 32 of 39 non-responsive patients have non-decreasing autoantibodies after or undetectable autoantibodies before rituximab treatment (Figure 1). A possible explanation for nondecreasing antibodies could be insufficient eradication of antibody producing plasma cells. Indeed long-lived plasma cells were detected in the spleen of ITP patients non responsive to rituximab treatment and it has been suggested that B cell depletion promotes the differentiation and settlement of these long-lived plasma cells in the spleen.15 Although nonresponsive patients without detectable platelet associated autoantibodies, fit the ASH criteria for ITP, these patients might represent a subgroup of ITP patients with possibly T-cell mediated platelet destruction. Indeed, Audia et al. in this respect showed the importance of activated splenic CD8+ T cells in ITP patients unresponsive to rituximab.16 Furthermore, CD8+ T cells were shown to contribute to the murine splenocyte's ability to induce thrombocytopenia; recently Arthur et al. showed CD8+ T cell mediated antibody-independent platelet clearance in a murine model for refractoriness to platelet transfusions.17, 18

Immune suppressive treatment in combination with Rituximab, as recently published, may improve the limited response for rituximab as single treatment.19 In this respect it will be interesting to study the effect of combination therapy in ITP patients with and without detectable platelet autoantibodies.

In conclusion, our results show absence of platelet-bound antibodies to be associated with a low response to rituximab. Additionally, response to rituximab appeared strongly associated with a decline in platelet-bound antibodies. Our findings importantly implicate that both antibody-negativity vs. strong antibody presence might enable a more individualized therapeutic approach in this group of ITP patients.

Appendix: study group members

The members of the Dutch HOVON 64 Study Group are: Jaap J. Zwaginga (Department of Immunohematology and Blood Transfusion, Leiden University Medical Centre and the Centre for Clinical Transfusion Research, Sanquin-Leiden University Medical Centre, Leiden, The Netherlands), Bronno van der Holt (HOVON Data Center, Erasmus MC Cancer Institute–Clinical Trial Centre, Rotterdam, The Netherlands), Peter A. te Boekhorst (Department of Hematology, Erasmus MC, Rotterdam, The Netherlands), Bart J. Biemond (Department of Hematology, Academic Medical Centre Amsterdam, Amsterdam, The Netherlands), Mark-David Levin (Department of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, The Netherlands), Rene van der Griend (Department of ´ Internal Medicine, Diakonessenhuis, Utrecht, The Netherlands), Anneke Brand (Department of Immunohematology and Blood Transfusion, Leiden University Medical Centre and the Centre for Clinical Transfusion Research, Sanquin-Leiden University Medical Centre, Leiden, The Netherlands), Sonja Zweegman (Department of Hematology, VU University Medical Centre, Amsterdam, The Netherlands), Hans F. M. Pruijt (Department of Internal Medicine, Jeroen Bosch Hospital, Den Bosch, The Netherlands), Vera M. J. Novotny (Department of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands), Art Vreugdenhil (Department of Internal Medicine, Maxima Medical Centre, Veldhoven, The ´ Netherlands), Marco R. de Groot (Department of Internal Medicine, Medisch Spectrum Twente, Enschede, The Netherlands), Okke de Weerdt (Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, The Netherlands), Elisabeth C. M. van Pampus (Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands), Tanja M. van Maanen-Lamme (Department of Internal Medicine, Westfriesgasthuis, Hoorn, The Netherlands), Shulamiet Wittebol (Department of Internal Medicine, Meander Hospital, Amersfoort, The Netherlands), Martin R. Schipperus (Department of Internal Medicine, HagaZiekenhuis, Den Haag, The Netherlands), Matthijs H. Silbermann (Department of Internal Medicine, Tergooiziekenhuizen, Blaricum, The Netherlands), Peter C. Huijgens (Department of Hematology, VU University Medical Centre, Amsterdam, The Netherlands), Marleen Luten (HOVON Data Center, Erasmus MC Cancer Institute–Clinical Trial Centre, Rotterdam, The Netherlands), Rene Hollestein (HOVON Data Center, Erasmus MC Cancer Institute–Clinical Trial Centre, Rotterdam, The Netherlands), Jan A. C. Brakenhoff (Department of Internal Medicine, Waterland Hospital, Purmerend, The Netherlands), Jolanda G. Schrama (Department of Internal Medicine, Spaarne Hospital, Hoofddorp, The Netherlands), Fransje A. A. Valster (Department of Internal Medicine, Lievensberg Hospital, Bergen op Zoom, The Netherlands), Gerjo A. Velders (Department of Internal Medicine, Gelderse Vallei Hospital, Ede, The Netherlands), and Harry R. Koene (Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, The Netherlands).

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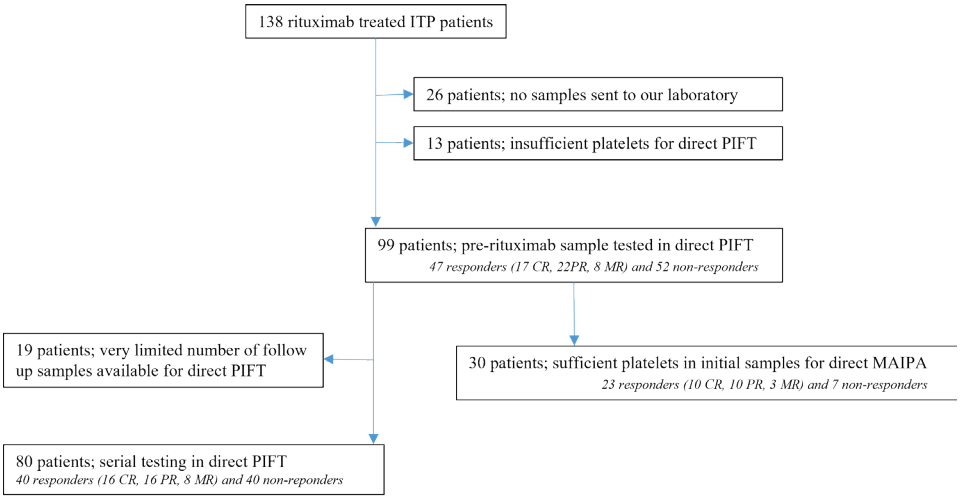
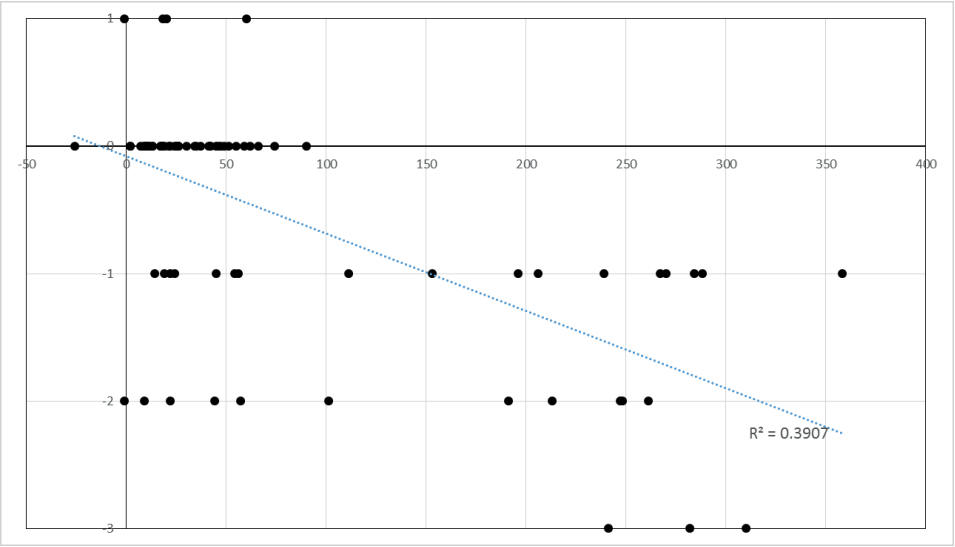
Supplemental data:



Supplemental Figure 1: *Of the 138 rituximab treated ITP patients included in the HOVON64 study, 99 pre-rituximab blood samples could be tested in the direct PIFT and 30 samples also in the direct MAIPA. For 80 patients sufficient follow up samples were available to perform serial testing in de direct PIFT. PIFT=Platelet Immunofluorescence Test, MAIPA=Monoclonal Antibody Immobilization of Platelet Antigens assay, CR=complete responder, PR=partial responder, MR=moderate responder, NR=non-responder*



Supplemental Figure 2: *Delta values, i.e. highest platelet count within ten weeks after rituximab treatment minus platelet count before rituximab treatment, compared with the corresponding direct PIFT result after rituximab treatment minus the result before treatment with rituximab. Spearman's correlation p<0.0001.*