Title of project:
Can 5B9, a monoclonal anti-PF4/heparin IgG that mimics human HIT antibodies, be used as an internal quality control for heparin-dependent platelet functional assays?

Short Name: 5B9 as IQC in HIT functional assays

Person responsible (Chair / Principal Investigator):
Prof Yves GRUEL/ Prof Andreas GREINACHER (co-investigator)

Description Abstract

State the application’s broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Suggested length is 2-3 paragraphs.

HIT is a rare and potentially fatal complication of heparin treatments, due to a specific immune response triggered by polyanion-modified PF4. Pathogenic antibodies are mainly IgG1, which activate platelets via FcγRIIA and the subsequent multicellular response is responsible for thrombotic complications. HIT is a clinicopathological syndrome and its diagnosis therefore depends on the presence of both clinical (i.e. thrombocytopenia, arterial or venous thrombosis…) and laboratory criteria. Two different categories of laboratory tests are available: immuno-assays (IAs), which detect antibodies against modified PF4, regardless of their ability to activate cells, and heparin-dependent functional assays. The main advantage of IAs is their excellent negative predictive value (nearly 100%), but at the price of a low positive predictive value (40 – 60%). However, combining an IA with a functional (platelet activation) test often improves the diagnostic accuracy of HIT.

Functional tests such as the 14C-serotonin release assay (14C-SRA) and heparin-induced platelet activation (HIPA) are considered gold standards, and are performed on washed platelets, but require highly specialized laboratories. Other more accessible platelet activation assays have been developed, such as flow cytometry (FC) assessing the expression of platelet membrane markers, or impedance aggregometry (HIMEA) performed on whole blood. Functional assays performed with platelet-rich plasma (PRP) or whole blood are less sensitive than methods using washed platelets such as SRA or HIPA, with high rate of false-negative results (1).
The reactivity of platelets from different donors to HIT antibodies is highly variable and there is currently no international standardized guideline for the appropriate selection of platelet donors for HIT functional testing. To select platelet donors, some laboratories use the anti-CD9 monoclonal antibody ALB6 that cross-links the FcRIIa receptor but this approach is not appropriate because ALB6 is murine and its affinity for FcgRIIa is different from that of a human IgG. Thus, in order to validate a technique and to evaluate the platelet reactivity, some laboratories test plasma samples from patients with confirmed HIT (2).

It is also important to note that there is still no IQC for platelet activation assays used for HIT diagnosis and for selecting the good platelet responders.

We recently developed in our laboratory the first chimeric monoclonal HIT antibody with a human Fc fragment, which behaves in a similar way to human IgG antibodies against PF4/heparin (3).

In this context we propose to evaluate whether the monoclonal antibody (5B9) can be used as positive control for HIT platelet activation assays and for platelet donor selection.

Design and methodology (Data expected to collect, sample size and statistical analysis):

Describe concisely the research design and methods for achieving these goals. Suggested length 2-3 paragraphs

The objective is to validate the use of 5B9 as an Internal Quality Control (IQC) for HIT functional assays.

At least one among five different functional assays (SRA, PAT, HIPA, HIMEA, FC) will be performed by each participating center, with 5 concentrations of 5B9 tested in the same experiment, according to the procedure currently used when they are evaluating patient samples (heparin concentration, dilution and platelet volume, incubation time...) for the diagnosis of HIT.

Each functional assay will be evaluated in at least 3 different laboratories.

- Vials of lyophilized 5B9 (500 µg) will be sent to each investigator center.
- 5B9 will be extemporaneously solubilized with distilled water (500 µL).
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- 5B9 will be tested at 5 different final concentrations: 1 – 10 – 20 – 50 – 100 µg/ml.

- The dilutions of 5B9 will be performed in normal human serum or plasma according to the protocol of each assay.

- Each functional assay will be evaluated with 5B9 by testing **10 different unselected platelet donors**.

- 5B9 concentrations will be tested in the absence or in the presence of low and high heparin concentrations (UFH or LWMH as usually employed). Heparin concentrations tested will be the same than those usually used by the investigator center with samples from patients with suspected HIT.

- The criteria of positivity and negativity must be clearly defined by each investigator. If applicable, the origin of the negative and/or positive controls used must be mentioned in the procedure.

- Each center will prepare platelets or whole blood according their usual procedure.

- The participating center will complete a data sheet with the final conclusion of the test performed with each donor and each 5B9 concentration. The results obtained for each heparin concentration tested will also be provided.

- Raw data will have to be kept by the centers.

- The agreement analyses will be done with the Kappa test ratio and the percentage of positive results will be compared with the Chi-2 test. A p value < 0.05 will be considered as significant.

**Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):**

Suggested length 2-3 paragraphs

- The choice of participating institutions was based on their experience in the field of HIT diagnosis.
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- Each participating center (listed in table below) will perform at least one or two platelet functional tests (SRA, HIPA, PAT, HIMEA, and flow cytometry) routinely used to confirm the diagnosis of HIT.

<table>
<thead>
<tr>
<th>Name of physician</th>
<th>Institution</th>
<th>Country</th>
<th>Test 1</th>
<th>Test 2</th>
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<tbody>
<tr>
<td>Y. Gruel / C. Pouplard</td>
<td>Laboratory of Haemostasis, CHU Tours</td>
<td>France</td>
<td>FC</td>
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<tr>
<td>A. Greinacher</td>
<td>Institute of Immunology and Transfusion Medicine, Greifswald</td>
<td>Germany</td>
<td>HIPA</td>
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<tr>
<td>I. Nazy</td>
<td>Platelet Immunology Laboratory, McMaster Hospital, Hamilton</td>
<td>Canada</td>
<td>SRA</td>
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<tr>
<td>T. Bakchoul</td>
<td>Center for Clinical Transfusion Medicine, Tubingen</td>
<td>Germany</td>
<td>HIPA</td>
<td>FC</td>
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<tr>
<td>N. Ajzenberg</td>
<td>Laboratory of Haemostasis, APHP, Paris</td>
<td>France</td>
<td>HIPA</td>
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<tr>
<td>B. Chong</td>
<td>Department of Haematology, The St George Hospital, KOGARAH</td>
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<td>SRA</td>
<td>PAT</td>
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<td>MC. Kopp</td>
<td>Department of Haematology, Royal North Shore Hospital, Sydney</td>
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<td>F. Mullier</td>
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<td>B. Curtis</td>
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<td>SRA</td>
<td>FC</td>
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<tr>
<td>P. Gresele</td>
<td>Department of Medicine - Section of Internal and Cardiovascular Medicine, Perugia</td>
<td>Italy</td>
<td>PAT</td>
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</tbody>
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Expected timeline:

- Project stage/set up
  - Launch: January 2020
  - Duration: 6 months
  - Finalization/analysis: 2 months
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Reporting

Expected outcomes (ie. publications): First semester 2021

Publication type (SSC Communication, Guidance document or original article):

SSC communication and original article

Description of project set/up and management, needed infrastructure and resources (summary):

- 5B9 will be lyophilized by Stago and sent to each investigator center.
- The complete protocol of each assay associated with the results obtained with 5B9 will be sent to the lead investigator center.
- The data analysis will be carried out in Tours, under the supervision of Pr Claire Pouplard.

References:

