Clinical and Laboratory Diagnosis of Heparin-Induced Thrombocytopenia (HIT) in Children: a Prospective Pilot Study

SECTION 1.

Specific Aims

1. To describe an inception cohort consisting of pediatric patients undergoing cardio-pulmonary bypass surgery (CPB) prospectively recruited upon their admission to The Hospital for Sick Children (Sickkids®). Subjects will be investigated prospectively for heparin-induced thrombocytopenia (HIT) by both heparin-PF4 (H-PF4) antibodies detected by enzyme immunoassays (ELISA) and by a confirmatory serotonin-release assay (SRA).

2. To compare the specificities and sensitivities of two ELISA screening assays in children tested for HIT regarding the detection of H-PF4 antibodies; the former laboratory method will be using 3 immunoglobulin (Ig) subclasses (IgA, IgM, and IgG); the latter will be testing only the IgG subclass.

3. To evaluate the potential use of clinical predictive tools previously develop for the investigation of HIT in adults in a pediatric population (i.e., the 4T score and the score developed by Lillo-Le et al for patients post-CPB)

4. To stratify the HIT assay results by patient type (i.e., use of the PELOD clinical severity score), pre-test risk assessment by two previously described clinical scoring system, and correlate these with their respective clinical-laboratory outcomes (incidence of thrombocytopenia, thrombosis, changes in anticoagulation therapy, and mortality).

5. The compare the clinical (thrombosis and mortality rates) and laboratory (thrombocytopenia) outcomes of pediatric patients undergoing CPB with the results of H-PF4 antibodies detected only by ELISA methods.

Background

1.0 Introduction

In pediatric tertiary care hospitals, approximately 10% to 15% of patients are exposed to
heparin daily, as this drug remains the most widely used anticoagulant to date (i.e. patients undergoing cardiac catheterization, cardiopulmonary bypass surgery, or extracorporeal membrane oxygenator) [1]. Advantages of heparin include its rapid onset of action, simple laboratory monitoring, low cost, and availability of a perfect antidote [2]. However, exposure to this drug comes at a price as it may result in the development of antibodies against heparin when it binds to platelet factor-4, a secondary messenger stored on platelet α-granules. In adults, subsets of antibodies of the immunoglobulin G (IgG) subclass directed against the newly formed heparin-platelet factor-4 (H-PF4) complex are able to activate platelets; their presence in the circulation (seroconversion) results in a spectrum of findings ranging from lack of clinical consequences to thrombocytopenia with or without thrombosis in the context of a potentially devastating prothrombotic syndrome known as Heparin-induced Thrombocytopenia (HIT) [3-7]. The estimated incidence rate of thrombotic complications of HIT in adults is 50% [8] and when associated with thrombosis, it harbors 2-11% risk of limb amputation and 20-30% mortality rate [9,10].

Heparin Induced Thrombocytopenia is a clinic-pathologic syndrome defined by the presence of one or more clinical events (thrombocytopenia with or without thrombosis) temporally related to heparin administration (between 5 to 10 days after initial heparin exposure) and caused by H-PF4 antibodies with platelet-activating properties (Figure 1).

Only a small portion of patients who develop H-PF4 antibodies will have thrombocytopenia and, further, only a small number of those patients with thrombocytopenia will have a thrombotic complication. In adult clinical practice the diagnosis of HIT combines pretest clinical probability with the result of laboratory investigations [11-12]. For that purpose, pretest clinical scores have been developed, such as the “4T” score [13], or the score developed by Lillo-Le et al for patients post-CPB [14].

In children, the reported incidence of HIT varies between 0 and 2.3% [1,11-19]. Several factors contribute to this lack of clarity. Ten studies have investigated the frequency of HIT and/or antiheparin-PF4 antibodies in children with a great degree of heterogeneity in the patient types and testing methodology. Conversely, a recent prospective study revealed that exposure to heparin results in seroconversion in up to 52% of pediatric cardiovascular patients, including IgG, IgM, and IgA H-PF4 antibody titers [15].

HIT antibodies can be detected by two types of tests: enzyme immunoassays (EIAs) and platelet activation (functional) assays. The serotonin release assay (SRA), a functional assay, is regarded as the “gold standard” but is technically demanding and performed by few laboratories. The majority of studies have employed EIAs or heparin-induced platelet aggregometry (HIPA) with only two of the 10 aforementioned studies and 3 out of over 70 pediatric case reports [16,24] using the SRA. Furthermore, the majority of these studies have involved patients in the intensive care unit and/or following cardiovascular surgery. These are groups in whom multiple prothrombotic risk factors exist and heparin-dependent antibodies, thrombocytopenia and thrombotic complications are not uncommon, making it difficult to discern the actual causal role of HIT in regards to their clinical outcomes.

Leonardo R. Brandao
The Hospital for Sick Children
The costs of HIT are considerable, owing largely to prolonged hospitalization and the cost of alternative anticoagulants whose safety profile has not been properly studied. Thus, the potential for overdiagnosis of HIT lends increasing importance to the roles of clinical scoring systems, immunologic and functional assays in diagnosing HIT in children [25].

1.1. Preliminary Data and Rationale

A retrospective review from May 2006 to August 2008, was conducted at The Hospital for Sick Children, where a commercial PF4-dependent enzyme immunoassay from GTi® Diagnostics (Waukesha, WI, USA) has been used as its screening test for HIT. This EIA detects PF4-dependent antibodies of three immunoglobulin classes (IgG, IgA, IgM) against PF4/polyvinyl sulfonate. A positive result was based on the manufacturer recommended cutoff of optical density (OD)λ405 ≥0.40 units and every positive sera was referred to the McMaster Platelet Immunology Laboratory, Hamilton, Ontario for confirmatory testing by platelet serotonin release assay (SRA). A total of 77 EIAs performed in 50 patients (mean age: 84 months) were identified. No cases of laboratory confirmed HIT (positive EIA and SRA) were identified; 13 screening assays (EIAs) tested positive (positive EIA in 8 subjects), and 2 of the confirmatory SRA testing initially interpreted as inconclusive were subsequently found to be negative. Subjects were recruited from medical (PICU), neonatal (NICU), and cardiac intensive care unit (CCU), hemodialysis (HD), general surgery, and nephrology. It is important to note that the seroconversion rate report in children of diverse underlying conditions varies from 0-52%, being partially responsible for our findings. However, while this retrospective review included only
pediatric patients screened due to suspicion of HIT (mostly by presence of thrombocytopenia), potentially leading to patient selection bias, the lack of confirmed cases suggests that this condition may have been over-reported in the literature, since the “gold standard” laboratory method has never been routinely applied in a systematic manner in previously published studies. The clinical impact of an inappropriately diagnosed case is tremendous, involving the use of expensive alternative anticoagulants, lack of expertise in their use, and a high rate of complications. Hence, establishing the appropriate diagnosis of HIT in children is key. In addition to the lack of routine utilization of the laboratory “gold standard” method, a previously described clinical scoring system has never been prospectively investigated in children.

1.2. Study Methodology

Annually, approximately 650 children are submitted to open heart surgery at our institution, and the vast majority of patients are being operated due to congenital heart defect; amongst those, around 75% are being submitted to a second operation. Data will be obtained prospectively. All subjects admitted to The Hospital for Sick Children meeting inclusion criteria will be investigated for HIT by a H-PF4-dependent enzyme immunoassay (ELISA) (GTi® Diagnostics (Waukesha, WI, USA); and by a second H-PF4 ELISA assay (Aniara®); blue top tube: 1.8 to 2.7-ml). In case of any positive ELISA, a confirmatory SRA (McMaster Platelet Immunology Laboratory, Hamilton, Ontario; blue top tube: 2.7-ml) will be obtained. The health records’ charts of these individuals will be reviewed and relevant data will be extracted as summarized in Appendix-1 (data collection form; IRB approved).

*Inclusion criteria:* All children aged 3 months to 18 years: a) admitted to the Cardiac Intensive Care Unit after being submitted to their second CPB surgery; or b) post-CPB and being diagnosed with a thrombus AND exposed to heparin: B.I) *newly or remotely (>100 days) exposed to heparin*; B.II) minimum exposure to heparin ≥ 5 days. ELISA testing (both laboratory methods) will be obtained on days 0, 5, and 14 of heparin exposure, or in the last day of SH administration, if ≥ 5 days and < 14 days. All subjects will be followed for the next 30 days from their heparin exposure, or from their first positive EIA.

*Exclusion criteria:*: a) Subjects re-exposed to heparin (exposure ≤100 days) with ELISA positivity at study entry; b) subjects aged less than 3 months of age, given their decreased capacity of producing an effective antibody response.

*Study timeline:*

- **DAY 0:** Lab: CBC, ELISA
- **DAY 5:** Lab: CBC, ELISA, SRA (if + ELISA)
- **DAY 14:** Lab: CBC, ELISA, SRA (if + ELISA)
Projected cost per patient:

<table>
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<th>Laboratory</th>
<th>Individual cost</th>
<th>Number of tests</th>
<th>Total cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC</td>
<td>39 CAD</td>
<td>3</td>
<td>117 CAD (clinical – cost not included)</td>
</tr>
<tr>
<td>ELISA (GTI®)</td>
<td>80 CAD</td>
<td>2 *</td>
<td>160 CAD</td>
</tr>
<tr>
<td>ELISA (Aniara®)</td>
<td>In kind</td>
<td>2 *</td>
<td>In kind</td>
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<tr>
<td>SRA</td>
<td>150 CAD</td>
<td>1</td>
<td>In kind</td>
</tr>
<tr>
<td>Total cost</td>
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<td>-</td>
<td>160 CAD</td>
</tr>
</tbody>
</table>

*average per patient, considering seroconversion of 50%

TOTAL GRANT APPLICATION: 62 pts x 160CAD = CAD 9,920

1.3. Sample Size and Statistical Analysis

A total of 62 subjects will be included, distributed among the following underlying conditions: a) 56 cardiovascular (2nd. surgery); b) 6 cardiovascular re-operation with thrombosis. Sampling for each category was calculated based on their respective previously reported EIA positivity seroprevalence rates (e.g. cardiovascular re-op [50%]). Regarding enrollment, eligible subjects will be recruited consecutively from the approximately 650 children with congenital heart defects undergoing surgery, of which approximately 487 are undergoing their second procedure. Data analysis will be largely descriptive. For comparison of values between patients with positive and negative HIT ELISAs, \( \chi^2 \) testing will be used for categorical variables and two-tailed \( t \)-test for continuous variables, such as platelet count and OD value. Significance will be defined by a two-sided \( \alpha \) of 0.05. For comparison of values between ELISA methodologies, ELISA testing and SRA testing, and clinical predictive tools and clinical outcomes, \( \chi^2 \) testing will also be used. All statistical analysis will be performed using SAS® analytical software.

1.4 Potential Implications

This pilot study will analyze the potential correlation (or lack of) between:

1) The sensitivity and specificity of two different ELISA test for H-PF4 antibodies in children post-CPB. At this point, the hypothesis for this specific aim is that the IgG-based ELISA test will be more specific and therefore, have a better correlation with the “gold standard” results provided by the concomitantly obtained SRA test. **IMPACT:** to shift the use of ELISA screening tool to the more specific laboratory method.

2) Correlation between ELISA and SRA testing in children. This analysis should help on the clarification of the true incidence of HIT in children post-CPB. **IMPACT:** Because this is only a pilot study, this project may help prove the suspicion that a bigger prospective study comparing
ELISA and SRA methods is necessary in this population to establish the true incidence of HIT in this patient group.

3) Correlation between clinical predictive tools and clinical-laboratory outcome will evaluate the clinical role of these tools in children. **IMPACT**: to add the use of clinical predictive model to the application of laboratory screening method in children, potentially increasing their respective sensitivity, specificity, positive and negative predictive values.

1.5 **Available Facilities**

Regarding laboratory investigation including both ELISA testing and SRA testing, the coagulation laboratories at The Hospital for Sick Children (director at SickKids®: Dr. William Brien) and the McMaster Platelet Immunology Laboratory, Hamilton, Ontario (director at McMaster: Dr. Theodore Warketin) have agreed to participate in the study.
SECTION 2.


SECTION 3.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
BRANDÃO, Leonardo R.

POSITION TITLE
Assistant Professor of Pediatrics

eRA COMMONS USER NAME (credential, e.g., agency login)

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YYYY</th>
<th>FIELD OF STUDY</th>
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<tbody>
<tr>
<td>Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil</td>
<td>M.D</td>
<td>Dec./1990</td>
<td>Medicine</td>
</tr>
<tr>
<td>Masters in Clinical Epidemiology, Health Policy, Management and Evaluation</td>
<td>MSc</td>
<td>2008-Present</td>
<td>Clinical Epidemiology</td>
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A. Personal Statement

The goal of the proposed research is to investigate the prevalence of HIT in children post-CPB. Specifically, we plan to establish a comparison of two distinct screening laboratory methods (ELISA-based) in comparison to the more specific SRA laboratory methodology in children submitted to CPB. In addition, laboratory results will be correlated to clinical and laboratory outcomes. Secondly, the role of predictive clinical scoring tools for HIT will also be evaluated within this patient population. I have the expertise, leadership and motivation necessary to successfully contribute to the proposed work. I have a broad background in pediatric hematology and coagulation, with specific training and expertise in key research areas for this application. During fellowship training at St. Jude’s Children’s Research Hospital, I was actively involved in studying clinical coagulation laboratory tools, as well as in a gene therapy laboratory in the expression of antithrombin. Most importantly, my second fellowship training focusing in the area of pediatric coagulation (Weill Cornell University), allowed me to acquire the expertise in the management of both bleeding (development of bleeding scores, investigation of platelet function defects), and thrombosis (role of inherited thrombophilia in venous thrombosis) in children. I currently co-direct the Thrombosis Service at The Hospital for Sick Children, in Toronto, ON, Canada; the largest Canadian Pediatric center which offers its largest referral based-tertiary hospital in the country. Additionally, I am involved with clinical and translational research (8 projects: perfusion medicine [4]; cardiology [4]) in collaboration with the Cardiology service, which will allow us to identify every patient eligible for the study.
B. Positions and Honors

Positions
1991-1992  Mandatory Army Service, Sao Paulo, Brazil
1992-1995  Pediatric Residency, Instituto da Crianca do Hospital das Clinicas - ICr./HCFMUSP; Faculdade de Medicina da Universidade de Sao Paulo
1995-1997  Pediatric Hematology-Oncology Fellowship, Instituto da Crianca do Hospital das Clinicas - ICr./HCFMUSP; Faculdade de Medicina da Universidade de Sao Paulo
1997-2000  Pediatric Hematology-Oncology Fellowship, Saint Jude Children's Research Hospital, Memphis, TN, USA
2000-2002  Pediatric Residency, Emory University School of Medicine, Atlanta, GA, USA
2002-2004  Pediatric Hemostasis Fellowship, Weill Medical College of Cornell University, New York, NY, USA
2004-Present  Staff, The Hospital for Sick Children, Toronto, ON, Canada

Honours
Research Award - Pediatric Residency - Emory University School of Medicine, The Importance of Inherited Platelet Defects in Mild Bleeding Disorders in Children and Adolescents. 2001

Best abstract competition – top 10 clinical abstract finalist: 53rd Annual Meeting Society of Thrombosis and Haemostasis Research, Vienna, Austria. February 5th, 2009


The Alvin Zipursky Teaching Award, Division of Haematology/Oncology, Department of Paediatrics, University of Toronto, The Hospital for Sick Children. Toronto, Canada. May 29th, 2009.


Best abstract competition – top 3rd clinical abstract finalist: Joint German and Dutch Societies of Thrombosis and Haemostasis, Nuremberg, Germany. February 24th, 2010.

C. Selected Peer-reviewed Publications

Most relevant to the current application


**Additional recent publications of importance to the field (in chronological order)**


D. Research Support

**Ongoing Research Support**
McCrindle(PI)  07/01/09-06/30/10 *Prevalence and Characterization of Post-Thrombotic Syndrome after Pediatric Cardiac Surgery.* Brian W McCrindle, Cedric Manlhiot, Leonardo Brandão, Suzan Williams, Anthony K Chan, Colleen E. Gruenwald, Ines B Menjak: Labatt Family Heart Centre Innovation funds;

Gruenwald (PI)  07/01/10-06/30/12 *Antithrombin enhancement may improve anticoagulation efficiency in infants undergoing cardiopulmonary bypass for cardiac surgery.* Colleen E. Gruenwald, Cedric Manlhiot, Glen van Arsdell, Leonardo Brandão, Anthony Chan; Heart and Stroke Foundation  Role: CoPI

Brandao (PI)  07/01/09-present Eisai Inc. Local Principal Investigator. Leonardo Brandão, Suzan Williams: A Three-Month Prospective Open Label Study of Therapy with Fragmin® (Dalteparin Sodium Injection) in Children with Malignancies and Venous Thromboembolism (2009-present) Role: PI

**Completed Research Support**
Brandao (PI)  07/01/08-06/30/09 *Seed Research Grant.* Leonardo Brandão: Web-based Pediatric Thrombosis Registry (2008/09) Role: PI

Brandao (PI)  07/01/07-06/30/08 *Sanofi-Aventis Educational Grant.* Leonardo Brandão, Suzan Williams: Internal Education Grant for Thrombosis Team at The Hospital for Sick Children Role: PI

James (PI)  07/01/07-06/30/08 *The Prevalence of Symptomatic Pediatric VWD.* Paula James, Victor Blanchette, Margaret Rand, Leonardo Brandão: Care until Cure Research, Canadian Hemophilia Society (CHS) Role: CoPI

McCrindle (PI)  07/01/07-06/30/08 *Prevention, detection and management of thromboembolic complications after pediatric cardiac surgery: toward evidence-based clinical protocols.* Cedric Manlhiot, Colleen Gruenwald, Leonardo Brandão, Anthony K Chan, Steven Schwartz, Helen Holtby, Ben Sivarajan, Glenn Van Arsdell, Chris Caldarone, Nadia Clarizia, Lynn Crawford-Lean, Brian W. McCrindle: Labatt Family Heart Centre Innovation Funds  Role: CoPI

Abshire (PI)  07/01/02-06/30/05 *Mild Bleeding Disorders in Children and Adolescents.* Thomas Abshire, Leonardo Brandão: Center for Disease Control and Prevention (CDC), Association of Teachers for Preventive Medicine (ATPM) Role: CoPI

**SECTION 4** (To be added and sent on November 1st, 2010)
Appendix 1 – DATA COLLECTION FORM (Pages 5-8)

Study ID# ____________

1. Age at time of enrolment ☐ months / years (circle)

2. Gender ☐ Male ☐ Female

3. Primary diagnosis _____________________

4. Secondary diagnoses _____________________

5. Patient types:

☐ Cardiovascular surgery
☐ Intensive care unit Neonatal Pediatric (circle)
☐ General surgery
☐ Neurology/neurosurgery
☐ Orthopedic surgery
☐ Nephrology/Hemodialysis
☐ Other: _____________________

6. Prior heparin exposure:
☐ Last 30 days ☐ 31 to 100 days ☐ No

Type of exposure:

☐ Heparin infusion (e.g. clot prophylaxis/therapy)
☐ ECMO
☐ Cardiac bypass
☐ Dialysis
7. Therapy: start date  

☐ Heparin:  ☐ Therapy  ☐ Prophylaxis

☐ Heparin infusion (e.g. clot prophylaxis/therapy)
☐ ECMO
☐ Cardiac bypass
☐ Dialysis

☐ Heparin exposure interrupted: date  

☐ Heparin restarted after testing
☐ Permanent discontinuation

☐ Shift to LMWH: date  

☐ Enoxaparin
☐ Tinzaparin

☐ Alternative anticoagulant used

☐ Danaparoid
☐ Argatroban
☐ Lepirudin
☐ Fondaparinux

☐ Hemorrhagic event while on anticoagulation

Details:  

_____________________________________________________
_____________________________________________________
_____________________________________________________
8. **Outcome**
   - [ ] Death
   - [ ] Amputation

   Reported cause of death: ________________________________

9. **Platelet count: (monitoring)**

<table>
<thead>
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<tbody>
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<td>Baseline</td>
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<tr>
<td>Day 15</td>
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