

## Proteomic profiling for biomarker discovery in heparin-induced thrombocytopenia

Tracking no: ADV-2024-012782R1

Henning Nilius (Inselspital, Bern University Hospital, and University of Bern, Switzerland) Hind Hamzeh-Cognasse (French Blood Establishment (EFS) Auvergne-Rhone-Alpes, France) Janna Hastings (University of Zurich, Switzerland) Jan-Dirk Studt (University Hospital Zürich, Switzerland) Dimitrios Tsakiris (University Hospital Basel, Switzerland) Andreas Greinacher (University Medicine Greifswald, Germany) Adriana Mendez (, ) Adrian Schmidt (Municipal Hospital Zurich Triemli, Switzerland) Walter Wuillemin (Division of Hematology and Laboratory of Hematology, Switzerland) Bernhard Gerber (Oncology Institute of Southern Switzerland, Switzerland) Prakash Vishnu (St. Michael Medical Center, Virginia Mason Franciscan Health, United States) Lukas Graf (Centre for Laboratory Medicine St. Gallen, Switzerland) Johanna Kremer Hovinga (Department of Hematology and Central Hematology Laboratory, Switzerland) Tamam Bakchoul (Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty of Tuebingen, University Hospital of Tuebingen, Germany) Fabrice Cognasse (French Blood Establishment (EFS) Auvergne-Rhone-Alpes, France) Michael Nagler (Inselspital University Hospital, Center for Laboratory Medicine, Switzerland)

### Abstract:

New analytical techniques can assess hundreds of proteins simultaneously with high sensitivity, facilitating the observation of their complex interplay and role in disease mechanisms. We hypothesized that proteomic profiling targeting proteins involved in thrombus formation, inflammation, and the immune response would identify potentially new biomarkers for heparin-induced thrombocytopenia (HIT). Four existing panels of the Olink proximity extension assay covering 356 proteins involved in thrombus formation, inflammation, and immune response were applied to randomly selected patients with suspected HIT (confirmed HIT, n=32; HIT ruled-out, n=38; positive heparin/PF4 [H/PF4] antibodies, n=28). The relative difference in protein concentration was analyzed using a linear regression model adjusted for sex and age. To confirm the test results, soluble P-selectin was determined using ELISA in above mentioned patients and an additional second dataset (n=49). HIT was defined as a positive heparin-induced platelet aggregation test (HIPA; washed platelet assay). Among 98 patients of the primary dataset, the median 4Ts score was 5 in patients with HIT, 4 in patients with positive heparin/PF4 antibodies, and 3 in patients without HIT. The median OD of a polyspecific heparin/PF4 ELISA was 3.0, 0.9, and 0.3, respectively. Soluble P-selectin remained statistically significant after multiple test adjustments. The area under the receiver-operating-characteristics-curve was 0.81 for Olink and 0.8 for ELISA. Future studies shall assess the diagnostic and prognostic value of soluble P-selectin in the management of HIT.

**Conflict of interest:** COI declared - see note

**COI notes:** The institution of JKH received grant support, consultancy fees, or honoraria from SNSF, Baxter/Takeda, Bayer, CSL-Behring, NovoNordisk, Octapharma, Roche, SOBI, Roche, Sanofi, FOPH, and Swiss Hemophilia Society, outside of the current work. MN received research grants from Bayer Healthcare, Roche diagnostics, Siemens healthineers, Pentapharm, and Bühlmann laboratories, as well as lecture fees from Sysmex, Siemens healthineers, and Euroimmun, outside of the current work. AG reports personal fees from Aspen, grants from Ergomed, grants from Boehringer Ingelheim, personal fees from Bayer Vital, grants from Rovi, grants from Sagent, personal fees from Chromatec, personal fees from Instrumentation Laboratory, grants and personal fees from Macopharma, grants from Portola, grants from Biokit, personal fees from Sanofi-Aventis, grants from Blau Farmaceutics, grants from Prosensa/Biomarin, grants and other from DRK-BSD NSTOB, grants from DRK-BSD Baden-Württemberg/Hessen, personal fees from Roche, personal fees from GTH e.V., grants from Deutsche Forschungsgemeinschaft, grants from Robert-Koch-Institut, non-financial support from Veralox, personal fees from Dilaflo, non-financial support from Vakzine Projekt Management GmbH, grants from GIZ Else-Körner-Stiftung, non-financial support from AstraZeneca, non-financial support from Janssen Vaccines & Prevention B.V., personal fees from Takeda Pharma, personal fees from Falk Foundation e.V., grants from European Medicines Agency, personal fees from Mylan Germany, outside the submitted work; In addition, Dr. Greinacher has a patent Screening Methods for transfusion related acute lung injury (TRALI) with royalties paid to EP2321644, 18.05.2011, and a patent Verfahren und Vorrichtung zur Herstellung von Universalplasma. licensed to DE 10 2020 212 609 B3 2022.04.07. TB reports grant support, consultancy fees, honoraria, or support for attending meetings from DFG, Stiftung Transfusionsmedizin und Immunhämatologie e.V, DRK Blutspendedienst, Deutsche Herzstiftung, Ministerium für Wissenschaft, Forschung und Kunst Baden Württemberg, Gesellschaft für Thrombose- und Hämostaseforschung, Berufsverband Deutscher Internisten, CoaChrom Diagnostica GmbH, Robert Bosch GmbH, Ergomed, Bayer, Bristol-Myers Squibb, Doctrina Med AG, Leo Pharma GmbH, Schöchle medical education GmbH, Mitsubishi Tanabe GmbH, Novo Nordisk GmbH, Swedish Orphan Biovitrum GmbH. All other authors declare that no conflict of interest exists.

**Preprint server:** No;

**Author contributions and disclosures:** HN wrote the analysis plan, analyzed, and interpreted the data, and wrote the first manuscript draft. HHC and FC contributed to the design of the study, analyzed and interpreted data, provided infrastructure and reagents, and contributed to the first draft of the manuscript. JH contributed to the analysis plan and interpretation of data. JDS, AG, DAT, AM, WAW, AS, JAKH, BG, PV, TB, and LG collected data. MN designed and implemented the study, collected data, contributed to analysis plan and interpretation of data, and wrote the manuscript. All authors contributed to the interpretation of data, reviewed the manuscript critically, and approved the final version of the manuscript.

**Non-author contributions and disclosures:** No;

**Agreement to Share Publication-Related Data and Data Sharing Statement:** Detailed data can be obtained upon reasonable request from the corresponding author.

**Clinical trial registration information (if any):**

# Proteomic profiling for biomarker discovery in heparin- induced thrombocytopenia

Henning Nilius<sup>1,2\*</sup>, Hind Hamzeh-Cognasse<sup>3,4\*</sup>, Janna Hastings<sup>5,6</sup>, Jan-Dirk Studt<sup>7</sup>,  
Dimitrios A. Tsakiris<sup>8</sup>, Andreas Greinacher<sup>9</sup>, Adriana Mendez<sup>10</sup>, Adrian Schmidt<sup>11</sup>,  
Walter A. Wuillemin<sup>12</sup>, Bernhard Gerber<sup>13</sup>, Prakash Vishnu<sup>14</sup>, Lukas Graf<sup>15</sup>, Johanna A.  
Kremer Hovinga<sup>16</sup>, Tamam Bakchoul<sup>17</sup>, Fabrice Cognasse<sup>3,4</sup>, Michael Nagler<sup>1, 18</sup>

<sup>1</sup> Department of Clinical Chemistry, Inselspital University Hospital Bern, Bern, CH

<sup>2</sup> Graduate School for Health Sciences, University of Bern, Bern, CH

<sup>3</sup> French Blood Establishment (EFS) Auvergne-Rhone-Alpes, Saint-Etienne, FR

<sup>4</sup> University Jean Monnet, Mines Saint-Etienne, INSERM, U 1059 SAINBIOSE, Saint-Etienne, FR

<sup>5</sup> Institute for Implementation Science in Health Care, Faculty of Medicine, University of Zurich, CH

<sup>6</sup> School of Medicine, University of St. Gallen, CH

<sup>7</sup> Division of Medical Oncology and Hematology, University and University Hospital Zurich, Zurich, CH

<sup>8</sup> Diagnostic Haematology, Basel University Hospital, Basel, CH

<sup>9</sup> Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin Greifswald, Greifswald, DE

<sup>10</sup> Department of Laboratory Medicine, Kantonsspital Aarau, Aarau, CH

<sup>11</sup> Institute of Laboratory Medicine and Clinic of Medical Oncology and Hematology, Municipal Hospital Zurich Triemli, Zurich, CH

<sup>12</sup> Division of Hematology and Central Hematology Laboratory, Cantonal Hospital of Lucerne and University of Bern, Lucerne, CH

<sup>13</sup> Clinic of Hematology, Oncology Institute of Southern Switzerland, Bellinzona, CH

<sup>14</sup> Fred Hutchinson Cancer Center, University of Washington, Seattle, USA

<sup>15</sup> Cantonal Hospital of St Gallen, St Gallen, CH

<sup>16</sup> Departement of Hematology and Central Hematology Laboratory, Inselspital Bern University Hospital

<sup>17</sup> Centre for Clinical Transfusion Medicine, University Hospital of Tübingen, Tübingen, DE

<sup>18</sup> University of Bern, Bern, CH

\* Shared first author

## Keywords

heparin-induced thrombocytopenia; proteomics; biomarker; SELP protein; P-selectin; inflammation

## Data sharing statement

All data is available from the corresponding author upon reasonable request.

## 41 **Key points**

- 42 1. This is the first study to apply proteomic profiling to patients with suspected  
43 HIT, thus analyzing a large number of potential proteins.
- 44 2. Our analysis provided evidence supporting the potential of soluble P-selectin as  
45 a promising new biomarker in HIT.

46

## 47 Abstract

48 New analytical techniques can assess hundreds of proteins simultaneously with high  
49 sensitivity, facilitating the observation of their complex interplay and role in disease  
50 mechanisms. We hypothesized that proteomic profiling targeting proteins involved in  
51 thrombus formation, inflammation, and the immune response would identify  
52 potentially new biomarkers for heparin-induced thrombocytopenia (HIT). Four existing  
53 panels of the Olink proximity extension assay covering 356 proteins involved in  
54 thrombus formation, inflammation, and immune response were applied to randomly  
55 selected patients *with suspected HIT* (confirmed HIT, n=32; HIT ruled-out, n=38;  
56 positive heparin/PF4 [H/PF4] antibodies, n=28). The relative difference in protein  
57 concentration was analyzed using a linear regression model adjusted for sex and age.  
58 To confirm the test results, soluble P-selectin was determined using ELISA in above  
59 mentioned patients and an additional second dataset (n=49). HIT was defined as a  
60 positive heparin-induced platelet aggregation test (HIPA; washed platelet assay).  
61 Among 98 patients of the primary dataset, the median 4Ts score was 5 in patients  
62 with HIT, 4 in patients with positive heparin/PF4 antibodies, and 3 in patients without  
63 HIT. The median OD of a polyspecific heparin/PF4 ELISA was 3.0, 0.9, and 0.3,  
64 respectively. Soluble P-selectin remained statistically significant after multiple test  
65 adjustments. The area under the receiver-operating-characteristics-curve was 0.81 for  
66 Olink and 0.8 for ELISA. Future studies shall assess the diagnostic and prognostic  
67 value of soluble P-selectin in the management of HIT.

## 68 1 Introduction

69 Diagnostic workup, assessment of prognosis, and treatment monitoring of heparin-  
70 induced thrombocytopenia (HIT) are hampered by a lack of reliable and specific  
71 biomarkers. HIT is a severe adverse reaction to heparin, one of the most commonly  
72 used anticoagulants <sup>1</sup>. Exposure to heparin can trigger the formation of platelet-  
73 activating antibodies against a heparin-platelet factor 4 complex <sup>2-5</sup>. Paradoxically,  
74 these antibodies can induce a prothrombotic state, leading to severe  
75 thromboembolism, limb loss, and even death <sup>6</sup>. In contrast, patients suspected of  
76 having HIT are often treated with dangerous anticoagulants with a high bleeding risk,  
77 such as argatroban <sup>7-9</sup>. Thus, misdiagnosis of HIT has severe consequences, including  
78 increased morbidity and mortality due to over- or undertreatment <sup>10</sup>. Due to their  
79 limited availability and prolonged turnaround times, washed platelet activation assays,

80 which are regarded as the reference standard, are not suitable for use in the acute  
81 phase of HIT <sup>11,12</sup>. The commonly used heparin/PF4 (H/PF4) antibody assays, however,  
82 have limited specificity and, therefore, put the patient at risk of overtreatment <sup>13</sup>.  
83 Despite recent advancements, including automated H/PF4 antibody assays, prediction  
84 models, and machine-learning applications, there is still a diagnostic gap that needs to  
85 be addressed <sup>14-17</sup>. Therefore, new biomarkers are a promising tool to develop  
86 enhanced diagnostic tests for the diagnosis, prognosis or monitoring of HIT <sup>18</sup>.

87 New analytical techniques enable the simultaneous determination of hundreds of  
88 biomarkers with extremely high sensitivity <sup>19</sup>. Proteins are critical mediators in  
89 hemostasis mechanisms, contributing to immunological response and inflammation,  
90 and venous and arterial thromboembolism <sup>20</sup>. These techniques can help observing the  
91 interplay of protein-biomarkers and their role in the mechanism of HIT. Among these  
92 techniques, Olink's proximity extension assay (PEA; Uppsala, Sweden) for proteomic  
93 profiling stands out for its high sensitivity, low risk of interferences, low specimen  
94 volume, and the large number of biomarkers that can be determined simultaneously  
95 <sup>21</sup>. This powerful platform has already been used successfully to identify potential  
96 biomarkers for a range of diseases, including cardiovascular disease, inflammatory  
97 diseases, cancer, and infectious diseases <sup>22-25</sup>.

98 We hypothesize that the application of proteomic profiling using the Olink platform can  
99 identify novel biomarkers for the management of HIT, potentially enabling a more  
100 accurate diagnosis.

## 101 **Methods**

### 102 **Study design, setting, and population**

103 The present analysis was conducted in-line with a large prospective cross-sectional  
104 study. Three groups of patients were selected out of 120 patients recruited in line with  
105 the TORADI-HIT dataset <sup>16,26</sup>, or a preceding pilot study <sup>11,27</sup>: (a) confirmed HIT, (b)  
106 H/PF4 antibodies present but HIT ruled out, and (c) HIT ruled out, H/PF4 antibodies  
107 not present (Figure 1; primary dataset). Patients in each group were randomly  
108 selected. An additional, random sample of 50 patients was selected to confirm the  
109 findings in a second dataset. Overall inclusion criteria were: (1) suspected HIT: anti-  
110 heparin-pf4 (H/PF4) antibody assay ordered OR 4Ts score rated OR hematology  
111 consultancy service requested, (2) age  $\geq$  18 years, and (3) general informed consent.  
112 Exclusion criteria were (1) insufficient serum specimen, (2) insufficient clinical data,

113 and (3) did not pass Olink quality control. The TORADI-HIT study recruited patients  
114 from 11 study centers in Switzerland, Germany, and the USA <sup>16</sup>. Most patients were  
115 included in Inselspital, University Hospital of Bern, Switzerland. Biomarker discovery  
116 was done using Olink's proximity extension assay (356 different proteins). The results  
117 were verified using ELISA determinations of the proteins (in French Blood  
118 Establishment (EFS) Auvergne-Rhone-Alpes, and University Jean Monnet, Mines Saint-  
119 Etienne, INSERM, U 1059 SAINBIOSE laboratory). The appropriate ethical committee  
120 approved the final protocol. The study was conducted in accordance with the  
121 declaration of Helsinki.

## 122 **Definition of patient groups**

123 HIT was defined by a positive washed-platelet functional assay, specifically the  
124 heparin-induced platelet activation assay (HIPA) <sup>11,16,27</sup>. Multiple studies have  
125 demonstrated that washed platelet assays, such as the serotonin release assay and  
126 HIPA, exhibit high sensitivity and specificity and strong concordance with clinical HIT.  
127 Therefore, the American Society of Hematology (ASH) and the British Committee for  
128 Standards in Hematology recommend these assays as reference standards <sup>28,29</sup>.  
129 Patients with positive heparin/PF4 antibodies were defined by a positive immunoassay  
130 (ELISA) but a negative HIPA. HIT-negative patients were defined by a negative ELISA  
131 and a negative HIPA.

## 132 **Work-up and laboratory tests**

133 Detailed clinical and laboratory data including residual serum samples were collected  
134 at diagnosis following a pre-specified protocol. Serum samples were frozen at -80°C.  
135 HIPA and H/PF4 immunoassay was conducted within one week after arrival. The  
136 laboratory technicians were blinded to the results of the other test and to the clinical  
137 information.

138 For the HIPA, serum samples were incubated with 4 different washed platelet  
139 donations in the presence of (a) only buffer, (b) 0.2 IU/ml low molecular weight  
140 heparin, and (c) 100 IU/ml heparin. All details were published previously <sup>11,16,30</sup>. The  
141 test was considered positive if aggregation occurred within 30 min for at least two  
142 donors in the presence of 0.2 IU/ml low-molecular-weight heparin, but not in the  
143 presence of 100 IU/ml heparin. On each plate, positive and negative controls were  
144 also measured.

145 For the H/PF4 immunoassay, the polyspecific Lifecodes PF4 Enhanced (Immucor,  
146 Dreieich, Germany) was performed according to the manufacturer's instructions.  
147 Optical density > 0.5 was considered positive. The test was previously validated in our  
148 laboratory and external and internal quality controls were performed <sup>11</sup>.

## 149 **Proteomic profiling**

150 To assess the proteomic profile, four existing panels of Olink's (Olink Proteomics Inc.,  
151 Uppsala, Sweden) proximity extension assay (PEA) were performed by Olink Uppsala:  
152 "Cardiovascular II", "Cardiovascular III", "Immune response" and "Inflammation".  
153 These panels comprise 356 different proteins involved in thrombus formation and  
154 inflammation. A full list of all proteins can be found in supplementary table S3. In  
155 short, the PEA recognizes proteins by pairs of oligonucleotide-linked antibodies <sup>21</sup>. If  
156 the antibodies bind in proximity to each other the oligonucleotides hybridize, and a  
157 new PCR primer sequence is revealed. This DNA barcode is then amplified and  
158 detected via quantitative PCR. The cycle threshold value, which is inversely correlated  
159 to the protein concentration in the sample, is then normalized and transformed to an  
160 arbitrary unit called normalized protein expression (NPX) on a log 2 scale. The quality  
161 of the measurements is assured through multiple internal controls (incubation  
162 controls, extension controls, and detection controls) as well as sample controls (inter-  
163 plate and negative controls), details of which are described elsewhere <sup>31</sup>. This  
164 innovative technique has been successfully used to identify various key biomarkers in  
165 a broad range of diseases, including venous thromboembolism <sup>22-25,32</sup>. The proteins  
166 were then annotated with their corresponding gene using the human protein atlas  
167 project <sup>33</sup>.

## 168 **P Selectin ELISA technology assay**

169 The levels of soluble P-selectin (soluble CD62P; corresponding to SELP; minimum  
170 detectable concentration: 0.244 ng/mL) were quantified in serum samples using  
171 ELISA (IBL International, Hamburg, Germany). Absorbance at 450 nm (for serotonin,  
172 405 nm) was measured using an ELISA plate reader (Magellan Software, Sunrise TM,  
173 Tecan Group Ltd, Lyon, France). Results were normalized to  $2 \times 10^8$  platelets/ml and  
174 data were expressed in pg/mL <sup>34</sup>.

## 175 **Statistical analysis**

176 To explore the variability between the different patient groups, a principal component  
177 analysis (PCA) using single value decomposition and sparse least square analysis

178 (sPLS) was used. Additionally, to quantify the association between protein levels and  
179 the presence of HIT we fitted a linear model to the data using the “stats” package for  
180 R. In the model, the NPX value of the different proteins served as the dependent  
181 variable while the HIPA status was used as the independent variable. To account for  
182 physiological differences among the patients, the model was adjusted for age and sex.  
183 The Benjamini-Hochberg method was used to adjust the calculated p-values to  
184 account for multiple testing, setting the false-discovery rate at 5%. A heatmap  
185 showing the 50 most significantly changed proteins, as well as a volcano plot, were  
186 plotted. For the biomarker that showed the highest significance, we created boxplots  
187 by thrombosis status and compared the different groups using the Wilcoxon-Rank-  
188 Sum test. Finally, to determine the diagnostic usefulness of the biomarker, we  
189 performed a receiver-operator characteristics curve (ROC) analysis and calculated the  
190 area under the curve (ROC-AUC). Additionally, we performed a multivariable linear  
191 regression and ROC-analysis using thrombosis as the dependent variable. All analyses  
192 were done in R version 4.1.2.

193 The appropriate ethical committee approved the final protocol (Kantonale  
194 Ethikkommission Bern).

## 195 **Results**

### 196 **Patient characteristics**

197 Out of a random sample of 120 patients, 32 with confirmed HIT were included, 28  
198 with H/PF4 detected (without HIT), and 38 without HIT (Figure 1; primary dataset).  
199 Overall, 21 were excluded because of insufficient clinical data or leftover sample  
200 material; one sample did not pass Olinks quality control. The median 4Ts score was 5  
201 in patients with HIT (inter-quartile range [IQR] 4, 6), 4 in patients with positive H/PF4  
202 antibodies (IQR 3.75, 4), and 3 in patients without HIT (2, 4). The median H/PF4  
203 ELISA was 3.0 (2.4, 3.0) in patients with HIT, 0.9 (0.7, 1.5), and 0.3 (0.2, 0.3) in  
204 patients without. Detailed patient characteristics are given in Table 1. From the second  
205 dataset comprising 50 patients with suspected HIT, one was excluded because of  
206 insufficient data (Figure 1). Among these patients, 12 were HIT-positive, 16 were  
207 H/PF4 positive, and 21 were HIT-negative. Detailed data of this second dataset is  
208 available in Table S1 of the supplementary material.

## 209 **Proteomic profile**

210 The primary dataset was used for proteomic profiling. In PCA and sPLS, minor  
211 differences between HIPA-positive and HIPA-negative patients were observed.  
212 Overlapping clusters were interpreted as a consequence of low patient numbers and  
213 similar patient characteristics (patients *with suspected HIT*). Results of the PCA and  
214 sPLS are displayed in Figure S1 and S2 of the supplementary material, respectively.

215 Protein abundance analysis revealed a statistically significant association of 40  
216 proteins with HIT status (8 upregulated, 32 downregulated). Out of these proteins,  
217 soluble P-selectin remained statistically significant after multiple test adjustments  
218 (false discovery rate 5%;  $\lambda = 1.04$ , 95% CI 0.63, 1.45). A clustered heatmap is  
219 available in Figure 2 and a volcano plot showing adjusted p-values is available in  
220 Figure 3. Fold changes with adjusted p-values are available in the supplementary  
221 material.

## 222 **ELISA and additional analyses**

223 An ELISA was used to determine the serum soluble P-selectin levels both in the  
224 primary data set that underwent Olinks PEA and in an additional data set of 49  
225 patients suspected of having HIT. First, we analyzed the first data set and found the  
226 following median soluble P-selectin values: 25783 pg/ml (IQR: 21238, 27157) for  
227 patients without HIT, 29350 pg/ml (IQR: 22175, 36963) for patients with negative  
228 HIPA but positive immunoassay, and 38150 pg/ml (IQR: 33888, 42075) for patients  
229 with HIT. There was a statistically significant difference between all groups when  
230 compared to the patients without HIT (no HIT vs. Antibody positive:  $p = 0.02$ ; no HIT  
231 vs. HIT:  $p = <0.01$ ).

232 Interestingly, different results are seen when analyzing only patients with  
233 thromboembolism: 32423 pg/ml (IQR: 27342, 36437), 33450 pg/ml (IQR: 24900,  
234 34650;  $p$ -value = 0.73), and 37750 pg/ml (IQR: 35988, 42925;  $p$ -value = 0.13) for  
235 HIT negative, antibody positive, and HIT positive patients, respectively.

236 Similar results were obtained in the second, confirmatory dataset: 24147 pg/ml (IQR:  
237 19627, 24149) in patients without HIT, 31547 (IQR: 24057, 31342;  $p$ -value = 0.02)  
238 in patients with positive antibodies, and 35048 (IQR: 32038, 38087;  $p$ -value =  $<$   
239 0.01) in patients with HIT. In contrast, no significant differences were seen in patients  
240 with thromboembolism. Boxplots showing soluble P-selectin levels for both datasets  
241 combined are displayed in Figure 4.

242 ROC-analysis of the soluble P-selectin as measured by Olink in the first cohort showed  
243 a ROC-AUC of 0.81 (95% CI: 0.72, 0.90) Similar results were observed with the  
244 ELISA (ROC-AUC 0.80; both groups).

245 ROC-analysis of soluble P-selectin for detecting thrombosis showed a lower ROC-AUC  
246 of 0.65 (95% CI: 0.52, 0.77) for the Olink assay and 0.67 (95% CI: 0.55, 0.79) for  
247 the ELISA (Figure S3). An additional multivariable linear regression showed a  
248 significant association between P-selectin levels and the different patient groups, even  
249 when adjusting for the presence of thrombosis, age, and sex (Table S2).

## 250 Discussion

251 We applied the Olink PEA covering 356 proteins involved in thrombus formation,  
252 inflammation, and immune response to 98 randomly selected patients with suspected  
253 HIT and confirmed the results with an ELISA assay in the patients mentioned above  
254 and an additional dataset of 47 patients. Among 40 proteins that were statistically  
255 significantly associated with HIT status in protein abundance analysis, soluble P-  
256 selectin remained significant after multiple test adjustments. This association was  
257 confirmed in a ROC analysis in PEA and ELISA (0.80 and 0.81 respectively). This  
258 association was especially apparent in patients *without* thrombosis, suggesting  
259 potential usefulness in this group.

260 To our knowledge, this is the first investigation to apply the PEA technology to patients  
261 *with suspected HIT*, thus analyzing a large number of proteins potentially associated  
262 with immune-mediated thrombosis. Prior omics-based analyses primarily focused on  
263 genetic variants. Four genome-wide association studies (GWAS) investigated the risk  
264 factors for HIT and revealed genetic variants associated with various enzymes, the  
265 AB0 Complex, and distinct receptor proteins<sup>18,35-37</sup>. However, comprehensive studies  
266 including metabolomics, proteomics, and transcriptomics are still missing<sup>18</sup>.

267 Our study suggest that soluble P-selectin holds potential as a diagnostic marker for  
268 HIT. P-selectin is a glycoprotein that is expressed in platelets and endothelial cells and  
269 is involved in leukocyte adhesion and thrombocyte aggregation<sup>38</sup>. When platelets are  
270 activated, P-selectin is mobilized from the  $\alpha$  granules to the external membrane<sup>39</sup>. In  
271 recent years, this mechanism has been leveraged to develop flow cytometry-based  
272 tests for activated platelets in patients suspected of having HIT. However, the  
273 diagnostic performance of these tests is limited<sup>27,40</sup>. Besides, soluble P-selectin, which  
274 can be released into the bloodstream through proteolytic cleavage or alternative

275 splicing, has been shown to be elevated in various cardiovascular and thrombotic  
276 disorders, including myocardial infarction, venous thrombosis, and COVID-19-related  
277 thrombosis<sup>41-45</sup>. Thus, soluble P-selectin appears to be a general marker for platelet  
278 activation<sup>46</sup>. Moreover, CD62P-mediated cross-talk between the vessel wall, platelets,  
279 monocytes and neutrophils results in the activation of innate immune cells and an  
280 increase in the expression of tissue factor. This initial activation of immune cells has  
281 the effect of thrombus reinforcement and retardation of subsequent resolution  
282 processes<sup>32</sup>. Interestingly, our findings extend decades-old observations on increased  
283 values of soluble P-selectin in patients with HIT<sup>47-49</sup>. However, these studies have  
284 methodological limitations, and soluble P-selectin was not yet considered a biomarker  
285 for HIT.

286 Our study has several strengths. Most importantly, the patients were randomly  
287 selected from a population of *patients with suspected HIT*. This is closely resembling  
288 the target population for a potential diagnostic or prognostic test, including not only  
289 patients with confirmed HIT but also patients with H/PF4 antibodies, and patients  
290 without HIT but with similar presenting diseases. As a consequence, contrasts are less  
291 pronounced compared to healthy controls but correspond to realistic clinical settings.  
292 In addition, we analyzed a large number of proteins in a relatively large cohort.  
293 Besides, the results obtained with the PEA were confirmed with an independent  
294 analytical technique (Luminex) and in a second dataset. All these points contribute to  
295 the high validity of the study.

296 However, our study also has some limitations. Firstly, we excluded a certain proportion  
297 of patients due to incomplete clinical data or residual sample material. However, we  
298 consider these dropouts to be at least "*at random*," and thus unlikely to affect the  
299 results of the study. Secondly, the population was not consecutive because of the high  
300 costs of the PEA tests. We cannot fully exclude that a certain selection bias is present.  
301 One might additionally argue that a matching procedure according to age and sex  
302 would increase the validity of the results. To account for this, we included age and sex  
303 in the regression model. These limitations suggest that our results must be confirmed  
304 in an independent, larger cohort of consecutive patients. Such a diagnostic accuracy  
305 study would also have to be carried out with test systems that can be used in daily  
306 practice (e.g. CLIA). Another limitation is that we have used serum rather than  
307 plasma. However, this limitation is minimized due to the differential analysis of the  
308 various groups using the same sample preparation process. In addition, a polyspecific  
309 rather than a IgG-specific ELISA was employed. However, there are three reasons why

310 this point did not introduce bias: (1) it is not a diagnostic accuracy study in which the  
311 performance of current tests is used as a comparison, (2) several studies have shown  
312 that the correlation between polyspecific and IgG-specific immunoassay is very high  
313 <sup>13</sup>, and (3) this information is not included in the study either as an investigated  
314 variable or as an outcome variable.

315 Our data confirm that soluble P-selectin is a promising new biomarker in patients with  
316 HIT. This fits to our current understanding of the mechanism of HIT, which recognizes  
317 platelet activation as an important feature. The concept of soluble P-selectin as a  
318 general biomarker for platelet activation is supported by comparable observations in  
319 many other thromboembolic diseases. Soluble P-selectin might be included in future  
320 diagnostic decision support tools, thus adding information about platelet activation.  
321 The protein may be particularly useful in the diagnosis of HIT without thrombosis,  
322 which is a particularly challenging diagnostic situation. Furthermore, the differential  
323 concentration of soluble P-selectin in patients with and without thrombosis suggests  
324 its potential use as a prognostic marker. This is consistent with observations  
325 suggesting soluble P-selectin as a prognostic marker for thromboembolism in COVID  
326 <sup>45</sup>. However, our findings must be confirmed in future studies, prospectively including  
327 patients with suspected HIT.

328 In conclusion, our analysis of 356 proteins associated with thrombus formation,  
329 inflammation, and immune response in a representative study of patients with  
330 suspected HIT has provided evidence supporting the potential of soluble P-selectin as  
331 a promising new biomarker. As this was particularly apparent in patients without  
332 thrombosis, a potential application appears not only as a diagnostic but also as a  
333 prognostic biomarker. Nevertheless, further validation of our findings in diverse  
334 settings and populations is warranted, necessitating prospective studies that include  
335 patients with suspected HIT.

## 336 **Acknowledgments**

337 This study was supported by a research grant from the Swiss National Science  
338 Foundation (#179334), the International Society on Thrombosis and Haemostasis  
339 (<https://www.isth.org/page/toradihit>), and the CTU research grants (Clinical Trial Unit,  
340 Inselspital, University Hospital). We thank Justine Brodard for implementing the  
341 heparin-induced platelet activation at Inselspital, Vincent Benites and Laura Celeste  
342 Rotondo for performing all laboratory tests, Anja Stalder and Margret Bachmann-Mac  
343 Donald for study management, and residents at all study centers. The authors wish to

344 acknowledge those individuals who provided technical support throughout our  
345 investigations including Charles-Antoine Arthaud, Marie-Ange Eyraud, and Amelie Prier  
346 from the 'Etablissement Français du Sang (EFS) Auvergne-Rhone-Alpes', France.

## 347 **Authorship Contributions**

348 HN wrote the analysis plan, analysed, and interpreted the data, and wrote the first  
349 manuscript draft. HHC and FC contributed to the design of the study, analysed and  
350 interpreted data, provided infrastructure and reagents, and contributed to the first  
351 draft of the manuscript. JH contributed to the analysis plan and interpretation of data.  
352 JDS, AG, DAT, AM, WAW, AS, JAKH, BG, PV, TB, and LG collected data. MN designed  
353 and implemented the study, collected data, contributed to analysis plan and  
354 interpretation of data, and wrote the manuscript. All authors contributed to the  
355 interpretation of data, reviewed the manuscript critically, and approved the final  
356 version of the manuscript.

## 357 **Conflict of Interest Disclosures**

358 The institution of JKH received grant support, consultancy fees, or honoraria from  
359 SNSF, Baxter/Takeda, Bayer, CSL-Behring, NovoNordisk, Octapharma, Roche, SOBI,  
360 Roche, Sanofi, FOPH, and Swiss Hemophilia Society, outside of the current work. MN  
361 received research grants from Bayer Healthcare, Roche diagnostics, Siemens  
362 healthineers, Pentapharm, and Bühlmann laboratories, as well as lecture fees from  
363 Sysmex, Siemens healthineers, and Euroimmun, outside of the current work. AG  
364 reports personal fees from Aspen, grants from Ergomed, grants from Boehringer  
365 Ingelheim, personal fees from Bayer Vital, grants from Rovi, grants from Sagent,  
366 personal fees from Chromatec, personal fees from Instrumentation Laboratory, grants  
367 and personal fees from Macopharma, grants from Portola, grants from Biokit, personal  
368 fees from Sanofi-Aventis, grants from Blau Farmaceutics, grants from  
369 Prosensa/Biomarin, grants and other from DRK-BSD NSTOB, grants from DRK-BSD  
370 Baden-Württemberg/Hessen, personal fees from Roche, personal fees from GTH e.V.,  
371 grants from Deutsche Forschungsgemeinschaft, grants from Robert-Koch-Institut,  
372 non-financial support from Veralex, personal fees from Dilaflor, non-financial support  
373 from Vakzine Projekt Management GmbH, grants from GIZ Else-Körner-Stiftung, non-  
374 financial support from AstraZeneca, non-financial support from Janssen Vaccines &  
375 Prevention B.V., personal fees from Takeda Pharma, personal fees from Falk  
376 Foundation e.V., grants from European Medicines Agency , personal fees from Mylan

377 Germany, outside the submitted work; In addition, Dr. Greinacher has a patent  
378 Screening Methods for transfusion related acute lung injury (TRALI) with royalties paid  
379 to EP2321644, 18.05.2011 , and a patent Verfahren und Vorrichtung zur Herstellung  
380 von Universalplasma. licensed to DE 10 2020 212 609 B3 2022.04.07. TB reports  
381 grant support, consultancy fees, honoraria, or support for attending meetings from  
382 DFG, Stiftung Transfusionsmedizin und Immunhämatologie e.V, DRK Blutspendedienst,  
383 Deutsche Herzstiftung, Ministerium für Wissenschaft, Forschung und Kunst Baden  
384 Württemberg, Gesellschaft für Thrombose- und Hämostasieforschung, Berufsverband  
385 Deutscher Internisten, CoaChrom Diagnostica GmbH, Robert Bosch GmbH, Ergomed,  
386 Bayer, Bristol-Myers Squibb, Doctrina Med AG, Leo Pharma GmbH, SchöchI medical  
387 education GmbH, Mitsubishi Tanabe GmbH, Novo Nordisk GmbH, Swedish Orphan  
388 Biovitrium GmbH. All other authors declare that no conflict of interest exists.

## References

- 390 1. Greinacher A. Heparin-Induced Thrombocytopenia. Solomon CG, ed. *N Engl J Med*.  
391 2015;373(3):252-261. doi:10.1056/NEJMcp1411910
- 392 2. Arepally GM, Cines DB. Pathogenesis of heparin-induced thrombocytopenia. *Translational*  
393 *Research*. 2020;225:131-140. doi:10.1016/j.trsl.2020.04.014
- 394 3. Vayne C, Guéry EA, Rollin J, Baglo T, Petermann R, Gruel Y. Pathophysiology and  
395 Diagnosis of Drug-Induced Immune Thrombocytopenia. *JCM*. 2020;9(7):2212.  
396 doi:10.3390/jcm9072212
- 397 4. Chong BH. Evolving concepts of pathogenesis of heparin-induced thrombocytopenia:  
398 Diagnostic and therapeutic implications. *Int J Lab Hematology*. 2020;42(S1):25-32.  
399 doi:10.1111/ijlh.13223
- 400 5. Marchetti M, Zermatten MG, Bertaggia Calderara D, Aliotta A, Alberio L. Heparin-Induced  
401 Thrombocytopenia: A Review of New Concepts in Pathogenesis, Diagnosis, and Management.  
402 *JCM*. 2021;10(4):683. doi:10.3390/jcm10040683
- 403 6. Kuter DJ, Konkle BA, Hamza TH, et al. Clinical outcomes in a cohort of patients with  
404 heparin-induced thrombocytopenia. *Am J Hematol*. 2017;92(8):730-738. doi:10.1002/ajh.24759
- 405 7. Marchetti M, Barelli S, Gleich T, et al. Managing argatroban in heparin-induced  
406 thrombocytopenia: A retrospective analysis of 729 treatment days in 32 patients with confirmed  
407 heparin-induced thrombocytopenia. *Br J Haematol*. 2022;197(6):766-790. doi:10.1111/bjh.18120
- 408 8. Warkentin TE. How to dose and monitor argatroban for treatment of HIT. *Br J Haematol*.  
409 2022;197(6):653-655. doi:10.1111/bjh.18153
- 410 9. Nilius H, Kaufmann J, Cuker A, Nagler M. Comparative effectiveness and safety of  
411 anticoagulants for the treatment of heparin-induced thrombocytopenia. *American J Hematol*.  
412 2021;96(7):805-815. doi:10.1002/ajh.26194
- 413 10. Dhakal B, Kreuziger LB, Rein L, et al. Disease burden, complication rates, and health-care  
414 costs of heparin-induced thrombocytopenia in the USA: a population-based study. *The Lancet*  
415 *Haematology*. 2018;5(5):e220-e231. doi:10.1016/S2352-3026(18)30046-2
- 416 11. Brodard J, Alberio L, Angelillo-Scherrer A, Nagler M. Accuracy of heparin-induced platelet  
417 aggregation test for the diagnosis of heparin-induced thrombocytopenia. *Thrombosis Research*.  
418 2020;185:27-30. doi:10.1016/j.thromres.2019.11.004
- 419 12. Greinacher A, Amiral J, Dummel V, Vissac A, Kiefel V, Mueller-Eckhardt C. Laboratory  
420 diagnosis of heparin-associated thrombocytopenia and comparison of platelet aggregation test,  
421 heparin-induced platelet activation test, and platelet factor 4/heparin enzyme-linked immunosorbent  
422 assay. *Transfusion*. 1994;34(5):381-385. doi:10.1046/j.1537-2995.1994.34594249047.x
- 423 13. Nagler M, Bachmann LM, Ten Cate H, Ten Cate-Hoek A. Diagnostic value of  
424 immunoassays for heparin-induced thrombocytopenia: a systematic review and meta-analysis.  
425 *Blood*. 2016;127(5):546-557. doi:10.1182/blood-2015-07-661215
- 426 14. Marchetti M, Barelli S, Zermatten MG, et al. Rapid and Accurate Bayesian Diagnosis of  
427 Heparin-induced thrombocytopenia. *Blood*. Published online January 16, 2020: blood.2019002845.  
428 doi:10.1182/blood.2019002845
- 429 15. Raschke RA, Gallo T, Curry SC, et al. Clinical effectiveness of a Bayesian algorithm for the  
430 diagnosis and management of heparin-induced thrombocytopenia. *Journal of Thrombosis and*  
431 *Haemostasis*. 2017;15(8):1640-1645. doi:10.1111/jth.13758
- 432 16. Nilius H, Cuker A, Haug S, et al. A machine-learning model for reducing misdiagnosis in  
433 heparin-induced thrombocytopenia: a prospective, multicenter, observational study.  
434 *eClinicalMedicine*. 2023;55:101745. doi:10.1016/j.eclinm.2022.101745
- 435 17. Bankova A, Andres Y, Horn MP, Alberio L, Nagler M. Rapid immunoassays for diagnosis  
436 of heparin-induced thrombocytopenia: Comparison of diagnostic accuracy, reproducibility, and  
437 costs in clinical practice. Garcia De Frutos P, ed. *PLoS ONE*. 2017;12(6):e0178289.  
438 doi:10.1371/journal.pone.0178289

- 439 18. Giles JB, Miller EC, Steiner HE, Karnes JH. Elucidation of Cellular Contributions to  
440 Heparin-Induced Thrombocytopenia Using Omic Approaches. *Front Pharmacol.* 2022;12:812830.  
441 doi:10.3389/fphar.2021.812830
- 442 19. Hanash S. Disease proteomics. *Nature.* 2003;422(6928):226-232. doi:10.1038/nature01514
- 443 20. Vivanco F, ed. *Vascular Proteomics: Methods and Protocols.* Vol 1000. Humana Press;  
444 2013. doi:10.1007/978-1-62703-405-0
- 445 21. Carlyle BC, Kitchen RR, Mattingly Z, et al. Technical Performance Evaluation of Olink  
446 Proximity Extension Assay for Blood-Based Biomarker Discovery in Longitudinal Studies of  
447 Alzheimer's Disease. *Front Neurol.* 2022;13:889647. doi:10.3389/fneur.2022.889647
- 448 22. Arunachalam PS, Wimmers F, Mok CKP, et al. Systems biological assessment of immunity  
449 to mild versus severe COVID-19 infection in humans. *Science.* 2020;369(6508):1210-1220.  
450 doi:10.1126/science.abc6261
- 451 23. Narula S, Yusuf S, Chong M, et al. Plasma ACE2 and risk of death or cardiometabolic  
452 diseases: a case-cohort analysis. *The Lancet.* 2020;396(10256):968-976. doi:10.1016/S0140-  
453 6736(20)31964-4
- 454 24. Rozeman EA, Hoefsmit EP, Reijers ILM, et al. Survival and biomarker analyses from the  
455 OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma. *Nat Med.*  
456 2021;27(2):256-263. doi:10.1038/s41591-020-01211-7
- 457 25. Zhong W, Edfors F, Gummesson A, Bergström G, Fagerberg L, Uhlén M. Next generation  
458 plasma proteome profiling to monitor health and disease. *Nat Commun.* 2021;12(1):2493.  
459 doi:10.1038/s41467-021-22767-z
- 460 26. Hammerer-Lercher A, Nilius H, Studt JD, et al. Limited concordance of heparin/platelet  
461 factor 4 antibody assays for the diagnosis of heparin-induced thrombocytopenia: an analysis of the  
462 TORADI-HIT study. *Journal of Thrombosis and Haemostasis.* Published online May  
463 2023:S1538783623004300. doi:10.1016/j.jtha.2023.05.016
- 464 27. Brodard J, Benites V, Stalder Zeerleder D, Nagler M. Accuracy of the functional, flow  
465 cytometer-based Emo-Test HIT Confirm® for the diagnosis of heparin-induced thrombocytopenia.  
466 *Thrombosis Research.* 2021;203:22-26. doi:10.1016/j.thromres.2021.04.017
- 467 28. Cuker A, Arepally GM, Chong BH, et al. American Society of Hematology 2018 guidelines  
468 for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Advances.*  
469 2018;2(22):3360-3392. doi:10.1182/bloodadvances.2018024489
- 470 29. Watson H, Davidson S, Keeling D. Guidelines on the diagnosis and management of heparin-  
471 induced thrombocytopenia: second edition. *Br J Haematol.* Published online October 2012:n/a-n/a.  
472 doi:10.1111/bjh.12059
- 473 30. Greinacher A, Michels I, Kiefel V, Mueller-Eckhardt C. A rapid and sensitive test for  
474 diagnosing heparin-associated thrombocytopenia. *Thrombosis and Haemostasis.* 1991;66(6):734-  
475 736.
- 476 31. *Olink-Data-Normalization-White-Paper-v2.0.Pdf.*; 2021:1-8. Accessed March 4, 2024.  
477 <https://olink.com/content/uploads/2021/09/olink-data-normalization-white-paper-v2.0.pdf>
- 478 32. Iglesias MJ, Sanchez-Rivera L, Ibrahim-Kosta M, et al. Elevated plasma complement factor  
479 H related 5 protein is associated with venous thromboembolism. *Nat Commun.* 2023;14(1):3280.  
480 doi:10.1038/s41467-023-38383-y
- 481 33. Uhlén M, Fagerberg L, Hallström BM, et al. Tissue-based map of the human proteome.  
482 *Science.* 2015;347(6220):1260419. doi:10.1126/science.1260419
- 483 34. Nguyen KA, Hamzeh-Cognasse H, Palle S, et al. Role of Siglec-7 in apoptosis in human  
484 platelets. *PLoS One.* 2014;9(9):e106239. doi:10.1371/journal.pone.0106239
- 485 35. Giles JB, Rollin J, Shaffer CM, et al. Genome-Wide Association Study Identifies Variation  
486 in *ABO* As Risk Factor for Platelet Reactivity in Heparin-Induced Thrombocytopenia. *Blood.*  
487 2020;136(Supplement 1):38-39. doi:10.1182/blood-2020-139651
- 488 36. Witten A, Bolbrinker J, Barysenka A, et al. Targeted resequencing of a locus for heparin-  
489 induced thrombocytopenia on chromosome 5 identified in a genome-wide association study. *J Mol*  
490 *Med.* 2018;96(8):765-775. doi:10.1007/s00109-018-1661-6

- 491 37. Karnes JH, Cronin RM, Rollin J, et al. A genome-wide association study of heparin-induced  
492 thrombocyto - penia using an electronic medical record. *Thromb Haemost.* 2015;113(04):772-781.  
493 doi:10.1160/TH14-08-0670
- 494 38. Pan J, Xia L, McEver RP. Comparison of Promoters for the Murine and Human P-selectin  
495 Genes Suggests Species-specific and Conserved Mechanisms for Transcriptional Regulation in  
496 Endothelial Cells. *Journal of Biological Chemistry.* 1998;273(16):10058-10067.  
497 doi:10.1074/jbc.273.16.10058
- 498 39. Woltmann G, McNulty CA, Dewson G, Symon FA, Wardlaw AJ. Interleukin-13 induces  
499 PSGL-1/P-selectin-dependent adhesion of eosinophils, but not neutrophils, to human umbilical  
500 vein endothelial cells under flow. *Blood.* 2000;95(10):3146-3152. doi:10.1182/blood.V95.10.3146
- 501 40. Althaus K, Pelzl L, Hidiatov O, Amiral J, Marini I, Bakchoul T. Evaluation of a flow  
502 cytometer-based functional assay using platelet-rich plasma in the diagnosis of heparin-induced  
503 thrombocytopenia. *Thrombosis Research.* 2019;180:55-61. doi:10.1016/j.thromres.2019.05.016
- 504 41. Panicker SR, Mehta-D'souza P, Zhang N, Klopocki AG, Shao B, McEver RP. Circulating  
505 soluble P-selectin must dimerize to promote inflammation and coagulation in mice. *Blood.*  
506 2017;130(2):181-191. doi:10.1182/blood-2017-02-770479
- 507 42. Blann A. The adhesion molecule P-selectin and cardiovascular disease. *European Heart*  
508 *Journal.* 2003;24(24):2166-2179. doi:10.1016/j.ehj.2003.08.021
- 509 43. Pabinger I, Ay C. Biomarkers and Venous Thromboembolism. *ATVB.* 2009;29(3):332-336.  
510 doi:10.1161/ATVBAHA.108.182188
- 511 44. Ramacciotti E, Blackburn S, Hawley AE, et al. Evaluation of Soluble P-Selectin as a Marker  
512 for the Diagnosis of Deep Venous Thrombosis. *Clin Appl Thromb Hemost.* 2011;17(4):425-431.  
513 doi:10.1177/1076029611405032
- 514 45. Fenyves BG, Mehta A, MGH COVID-19 Collection & Processing Team, et al. Plasma P -  
515 selectin is an early marker of thromboembolism in COVID -19. *American J Hematol.* 2021;96(12).  
516 doi:10.1002/ajh.26372
- 517 46. Kosteljik EH, Fijnheer R, Nieuwenhuis HK, Gouwerok CWN, De Korte D. Soluble P-  
518 selectin as Parameter for Platelet Activation during Storage. *Thromb Haemost.* 1996;76(06):1086-  
519 1089. doi:10.1055/s-0038-1650710
- 520 47. Chong B, Murray B, Berndt M, Dunlop L, Brighton T, Chesterman C. Plasma P-selectin is  
521 increased in thrombotic consumptive platelet disorders. *Blood.* 1994;83(6):1535-1541.  
522 doi:10.1182/blood.V83.6.1535.1535
- 523 48. Fareed J, Walenga JM, Hoppensteadt DA, et al. Soluble Adhesion Molecules in the HIT  
524 Syndrome: Pathophysiologic Role and Therapeutic Modulation. *Clin Appl Thromb Hemost.*  
525 1999;5(1\_suppl):S38-S44. doi:10.1177/10760296990050S108
- 526 49. Amin HM, Ahmad S, Walenga JM, Hoppensteadt DA, Leitz H, Fareed J. Soluble P-Selectin  
527 in Human Plasma: Effect of Anticoagulant Matrix and its Levels in Patients With Cardiovascular  
528 Disorders. *Clin Appl Thromb Hemost.* 2000;6(2):71-76. doi:10.1177/107602960000600204
- 529

530 **Tables**

531

532 Table 1: Patient characteristics of the primary dataset

	HIT positive	H/PF4 positive	HIT negative
n	32	28	38
Male sex (%)	22 (68.8)	17 (60.7)	24 (63.2)
Age (median [IQR])	68.5 [64.8, 76.0]	77.0 [55.0, 79.0]	74.0 [54.0, 81.0]
4Ts (median [IQR])	5 [4, 6]	4 [4, 5]	3 [2, 4]
ELISA GTI polyspecific OD (median [IQR])	3.0 [2.4, 3.0]	0.9 [0.7, 1.5]	0.3 [0.2, 0.3]
Setting (%)			
Cardiac surgery	13 (40.6)	3 (10.7)	4 (10.5)
ICU	10 (31.2)	12 (42.9)	14 (36.8)
Others	9 (28.1)	13 (46.4)	20 (52.6)
Thrombocytes G/L (median [IQR])	60 [43, 81]	68 [48, 101]	59 [41, 80]
Thrombosis (%)	15 (57.7)	5 (25.0)	4 (11.8)

533

534 Figure 1: Flow of the patients (primary and second dataset)

535 Figure 2: Clustered heatmap illustrating the z-scores of the 50 most significant  
 536 proteins, stratified by HIT status (group). The following additional information  
 537 is shown: sex, setting, and presence of thrombosis.

538 Figure 3: Volcanoplot showing the differential abundance of proteins between  
539 between HIT-patients and non-HIT patients (including Heparin/PF4 antibody  
540 positives). The X-axis depicts the fold change (NPX difference) while the Y-axis  
541 depicts the  $-\log_{10}(\text{adjusted p-value})$ . Green dots represent a p-value  $< 0.05$ ,  
542 and red dots represents adjusted p-values between 0.05 and 0.3.

543 Figure 4: P-selectin in patients with HIT, positive heparin/PF4 antibodies, and  
544 without HIT, depending on the presence of thromboembolism (ELISA, all pa-  
545 tients)

546 Figure 5: Receiver-operating characteristic (ROC) curve of P-selectin for the  
547 presence of HIT as measured with the Olink assay and ELISA (all patients)

548

# Figure 1

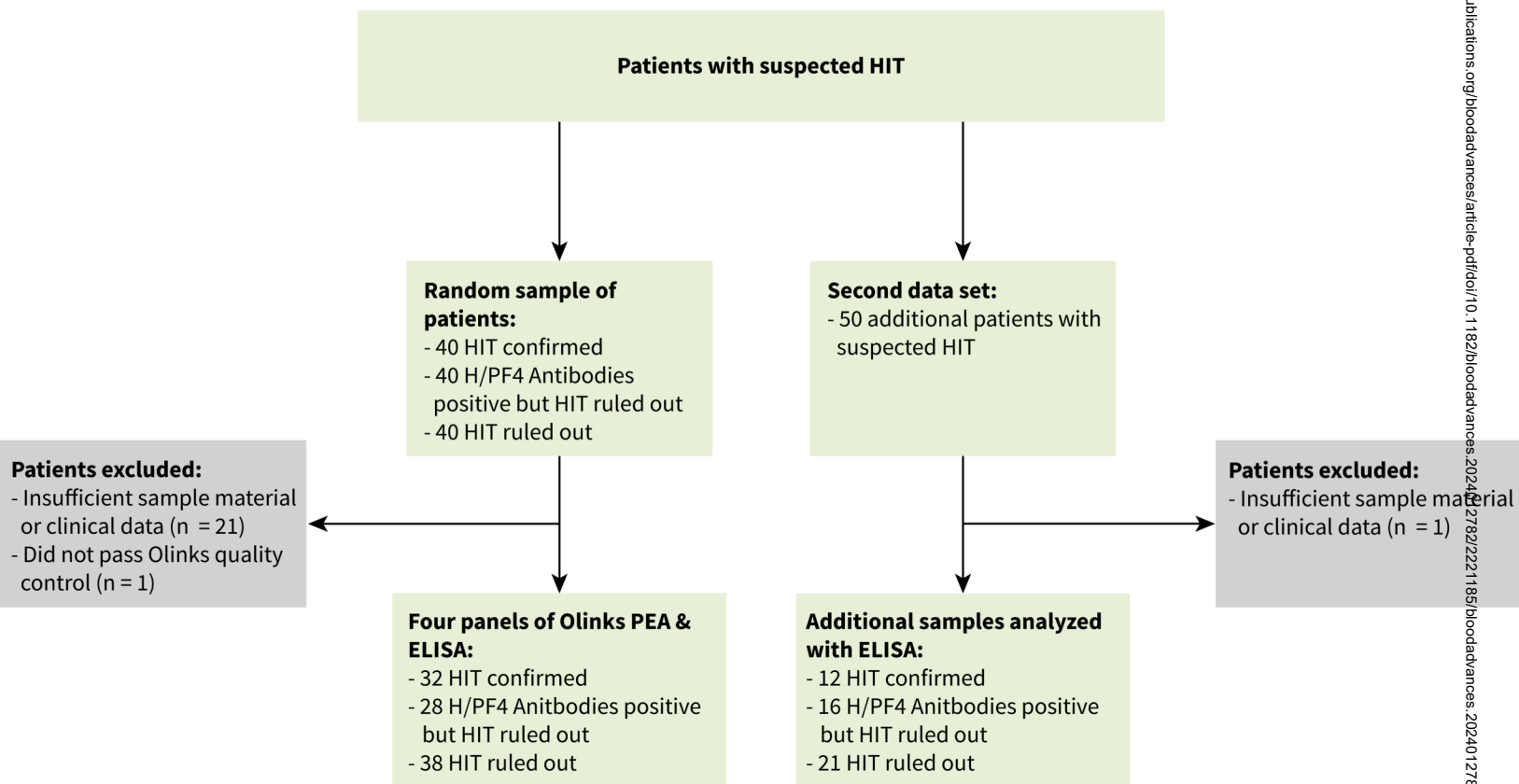


Figure 2

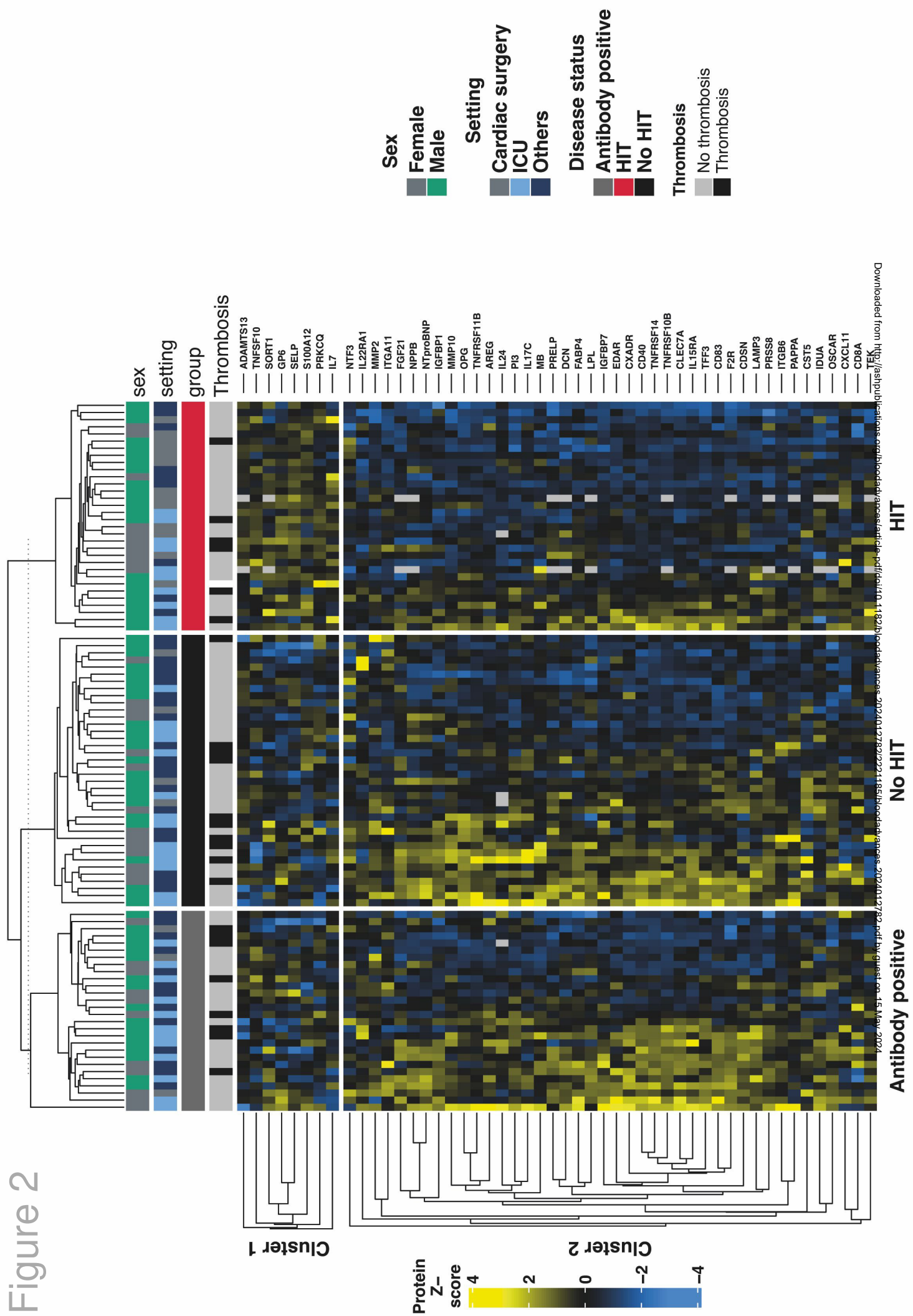


Figure 3



Figure 4

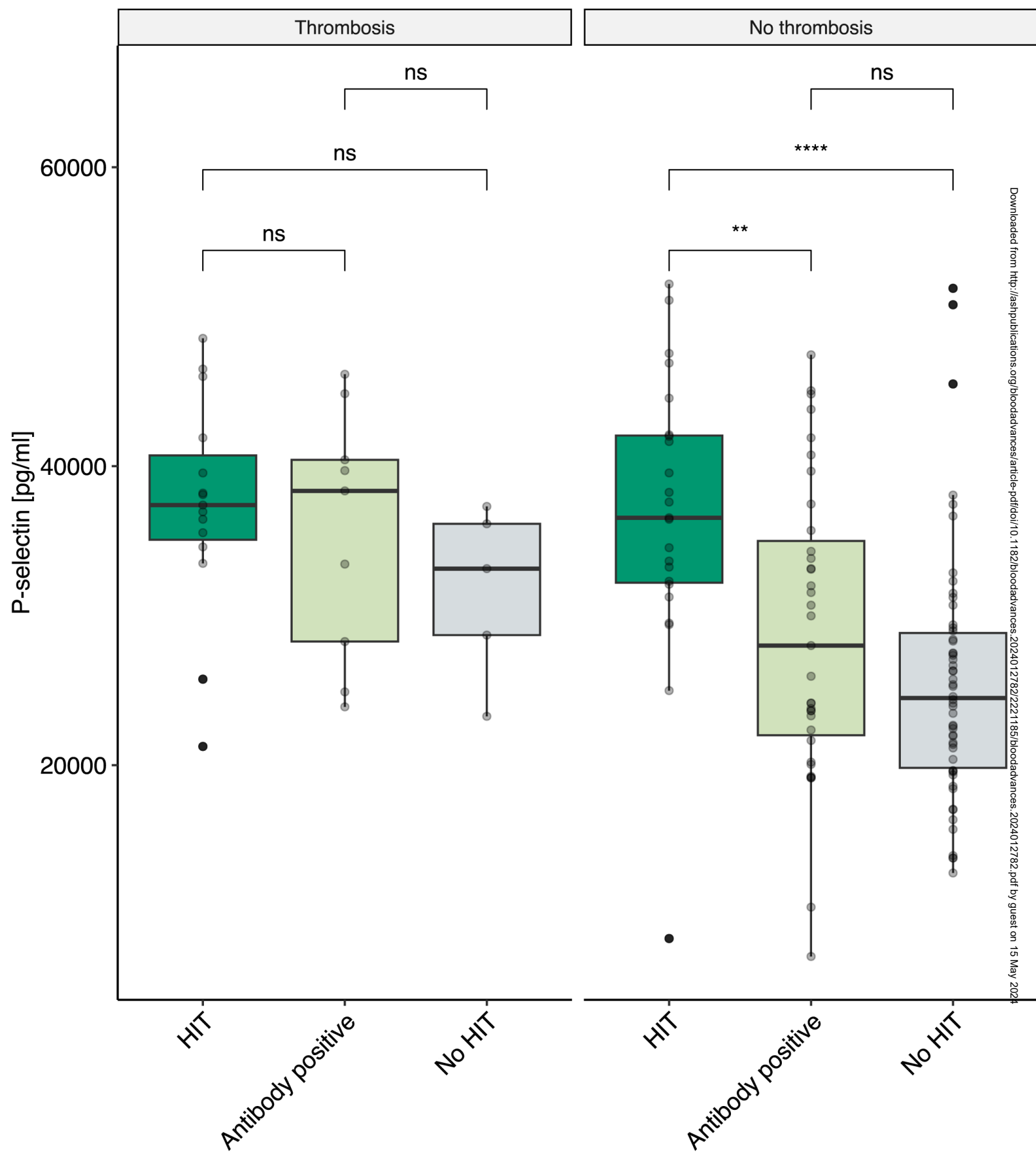


Figure 5

