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Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol

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1 Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease
2 in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol

3
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35 Abstract

37 Introduction

38 Hemorheological and hemostatic changes predispose to the development of arterial and
39 venous thrombotic events; however, limited information is available on the status of these
40 changes in celiac disease (CeD) and inflammatory bowel disease (IBD). In this study, we aim
41 to describe the hemorheological and hemostatic profiles of CeD and IBD patients in a
42 Hungarian cohort of patients to investigate whether any alterations contribute to elevated
43 thrombotic risk.

44 Methods and analysis

45 This is a case-control study involving newly diagnosed and followed CeD and IBD
46 patients with age- and sex-matched non-CeD, non-IBD subjects with an allocation ratio of
47 1:1:1.

48 After informed consent is obtained, a detailed medical history will be collected, including
49 venous and arterial thrombotic risk factors and medications. Symptoms in CeD patients will
50 be assessed with the Gastrointestinal Symptoms Rating Scale, and disease activity in IBD
51 patients will be determined by calculating the Mayo Score or Crohn's Disease Activity Index.
52 A single trained dietitian will assess dietary adherence among CeD patients with a thorough
53 interview together with a measurement of self-reported adherence, dietary knowledge, and
54 urine analysis (detection of gluten immunogenic peptides). In addition to routine laboratory
55 parameters, hemorheological (i.e., erythrocyte deformability and aggregation, viscosity of
56 whole blood and plasma) and hemostatic parameters (e.g., protein C, protein S, and
57 antithrombin) with immunological indicators (i.e., celiac-specific serology and
58 antiphospholipid antibodies) will be measured from venous blood for every participant.

59 Primary and secondary outcomes will be hemorheological and hemostatic parameters,
60 respectively. Univariate and multivariate statistics will be used to compare CeD and IBD
61 patients to control subjects. Subgroup analysis will be performed by disease activity.

62 Ethics and dissemination

63 The study was approved by the Regional and Local Research Ethics Committee,
64 University of Pécs (Ref No 6917). Findings will be disseminated at research conferences and
65 in peer-reviewed journals.

66 Trial registration

67 ISRCTN49677481.

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69 **Key words:** celiac disease, inflammatory bowel disease, hemorheology, thrombosis

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71 **Strengths and limitations of this study**

- 72 • Immune-mediated bowel diseases are associated with an increased risk of arterial and
73 venous thrombosis, but specific hemorheological and hemostatic alterations are
74 understudied in celiac disease and incomplete in inflammatory bowel disease.
- 75 • This case-control study prospectively recruits newly diagnosed and followed-up cases
76 of celiac disease and inflammatory bowel disease with age- and sex-matched controls
77 (the allocation ratio will be 1:1:1, respectively) to investigate clinical and laboratory
78 alterations predisposing to thrombosis.
- 79 • Laboratory tests include the measurement of hemorheological (i.e., erythrocyte
80 aggregation and deformability, plasma and whole blood viscosity), hemostatic
81 parameters (e.g., levels of fibrinogen, prothrombin time, protein C, protein S, and
82 antithrombin), and immunological indicators (e.g., celiac-specific serology and
83 antiphospholipid antibodies).
- 84 • Patients will be divided by disease activity into active and inactive.
- 85 • Results should be interpreted with caution due to the single-centre nature and case-
86 control design of the study.

88 **INTRODUCTION**

89 Immune-mediated disorders may affect 5–7% of the population.[1] These disorders
90 frequently share pathways in pathogenesis as well as organ manifestations. Celiac disease
91 (CeD) and inflammatory bowel disease (IBD) are systemic immune-mediated disorders,
92 primarily affecting the intestines.[2] Both are significant contributors to the load on
93 gastroenterological out-patient clinics due to various diagnostic issues and lifelong follow-
94 up.[3]

96 **CeD and IBD**

97 Global prevalence of biopsy-confirmed CeD is around 0.8%.[4] Genetic (HLADQ2 or
98 DQ8 haplotypes) and environmental factors (ingestion of gluten) play a crucial role in disease
99 development and during the course of the disease. Strict adherence to a lifelong gluten-free
100 diet (GFD) results in symptom relief and a significant reduction in disease complications.[5]

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3 101 IBDs are less frequent entities: the increasing prevalence of ulcerative colitis and Crohn's
4 102 disease may reach 0.5% and 0.3% in Europe, respectively.[6, 7] In addition to genetic
5 103 vulnerability, environmental factors and immune dysregulation are important contributors to
6 104 disease pathogenesis.[8] Although the pharmacological approach has been improved
7 105 significantly, treatment imposes a great burden on patients as well as on the healthcare
8 106 system.[7, 9]
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14 108 **Thrombosis and immune-mediated disorders**

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16 109 Immune-mediated disorders are often characterized by an increased risk of venous and
17 110 arterial thrombotic events.[10-14] The importance of these events is highlighted by the fact
18 111 that myocardial infarction, stroke, and venous thrombosis (deep venous thrombosis and
19 112 pulmonary embolism) are the three most common life-threatening cardiovascular
20 113 disorders.[15] Patients with atherosclerotic complications carry an increased risk of venous
21 114 thrombosis, and, conversely, venous thrombosis predisposes to the development of
22 115 atherosclerotic complications.[16-18] Mechanisms of thrombophilia in immune-mediated
23 116 disorders are complex, and acquired factors seem important.[19]
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29 117 Clinical presentation of CeD-associated hypercoagulability includes a wide variety of
30 118 thrombosis at venous sites, pulmonary embolism, atheroembolism (stroke), and obstetric
31 119 complications.[20] The multifactorial etiology of thrombosis may embrace the interplay of
32 120 malabsorption (vitamin and mineral deficiencies, e.g., vitamin B₁₂ and K deficiency),
33 121 thrombophilic autoantibodies (anti-tissue transglutaminase (tTG) and antiphospholipid
34 122 antibodies), hyperhomocysteinemia, endothelial dysfunction, accelerated atherosclerosis,
35 123 thrombocyte dysfunction, and genetics.[20-24] Immune-mediated comorbidities
36 124 ('autoimmune traits'), such as antiphospholipid syndrome, may contribute to the elevated
37 125 thrombotic risk as well.[24] In addition, ingestion of trace amounts of gluten may maintain a
38 126 continuous pro-inflammatory response.[25]
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45 127 IBD is associated with venous thrombosis and pulmonary embolism as well as with the
46 128 cardiovascular consequences of atherosclerosis, i.e., stroke and myocardial infarction.[26, 27]
47 129 The increased risk of thrombosis in IBD can be attributed to immobilization, surgical
48 130 interventions, glucocorticoid therapy, vitamin deficiencies, hyperhomocysteinemia, and
49 131 chronic inflammation alone or in conjunction with the factors above.[28, 29] In the case of
50 132 IBD, disease activity may be a crucial determinant of thrombotic risk.[27]
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56 134 **Thrombosis in hemorheological and hemostatic aspects**

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3 135 Rheological properties of blood can be described by specific laboratory measures,
4 136 including plasma viscosity, blood viscosity, erythrocyte deformability, and erythrocyte
5 137 aggregability. An altered hemorheological profile contributes to the development of arterial
6 138 thrombotic events, such as myocardial infarction and stroke.[30-33] In addition, red blood
7 139 cells and fibrinogen are suspected to be involved in the formation of venous thrombi ('red
8 140 clots').[34]

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12 141 Epidemiological studies indicate that arterial and venous coagulopathies are frequently
13 142 associated with altered levels or function of pro- and anticoagulant proteins (including
14 143 antithrombin, protein C, and protein S), altered activity of clotting factors, abnormal thrombin
15 144 generation, and endothelial damage.[35]

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19 145 Reports indicate that immune-mediated disorders may be associated with
20 146 hemorheological[36-38] and hemostatic changes[39-41], thereby contributing to the increased
21 147 risk of thrombotic events.
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25 149 **Prothrombotic hemorheological and hemostatic changes in CeD and IBD**

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27 150 No studies have assessed the hemorheological changes in CeD. There are sporadic reports
28 151 on activity-dependent prothrombotic hemorheological changes in IBD. However, while
29 152 individual studies have focused on single outcomes of laboratory parameters, none of them
30 153 have assessed the complete hemorheological profile of patients.[42-45]

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32
33 154 There has only been one retrospective publication that examined the link between CeD and
34 155 hemostatic alterations in a small cohort of patients: sporadic cases of protein C and protein S
35 156 deficiency (due to vitamin K malabsorption), hyperhomocysteinemia, and antiphospholipid
36 157 antibodies were identified.[22] In IBD patients, a significant decline in anticoagulant
37 158 mechanism is well-established.[46-49]

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42 160 **Scope and objectives**

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45 161 No studies have assessed hemorheological and hemostatic parameters within a study to
46 162 provide an overall view of thrombotic risk. Since our knowledge of hemorheological and
47 163 hemostatic changes is limited in CeD and IBD, this study aims to carry out a comprehensive
48 164 evaluation of venous and arterial prothrombotic alterations in these pro-inflammatory diseases
49 165 in a Hungarian cohort of patients.

50 166 1. Primary objective

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 - to assess the hemorheological profile of CeD and IBD patients, compared to non-

55 168 CeD, non-IBD subjects

169 2. Secondary objective

- 170 • to assess the hemostatic profile of CeD and IBD patients, compared to non-CeD,
171 non-IBD subjects

172 Our results can contribute to expanding our knowledge on the prothrombotic
173 pathophysiological alteration in CeD and IBD, thereby providing the basis for future research
174 on the indications of thromboprophylaxis under special prothrombotic circumstances, such as
175 hospitalization, pregnancy, or immobilization.

176

177 **METHODS AND ANALYSIS**

178

179 **Design**

180 This is a case-control study with prospective recruitment of CeD and IBD patients with
181 non-CeD, non-IBD control subjects. The study does not change the routine management of
182 subjects included (for the World Health Organization checklist, see Table 1). The study
183 protocol was planned in accordance with the SPIRIT 2013 Statement.[50]

184

185 Table 1. World Health Organization checklist

Data category	Information
Primary registry and trial identifying number	ISRCTN49677481
Date of registration in primary registry	05/03/2018
Secondary identifying numbers	None
Source(s) of monetary or material support	University of Pécs Medical School; Momentum Grant from the Hungarian Academy of Sciences (LP2014-10/2014); Highly Cited Publication Grant (KH 125678) from the National Research Development and Innovation Office; GINOP 2.3.2-15-2016-00048 Stay Alive and EFOP 3.6.2-16-2017-00006 Live Longer; Translational Medicine Foundation; and New National Excellence Programme, Ministry of Human Capacities (ÚNKP-17-3-II).
Primary sponsor	None
Secondary sponsor(s)	None
Contact for public queries	Zsolt Szakács, MD, szakacs.zsolt@pte.hu
Contact for scientific queries	Judit Bajor, MD, bajor.judit@pte.hu
Public title	Investigation of hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with healthy subjects: A case-control study (HERMES)
Scientific title	Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects: A case-control study (HERMES)
Countries of recruitment	Hungary

Health condition(s) or problem(s) studied	Celiac disease and inflammatory bowel disease
Intervention(s)	Questionnaires (thrombophilia, dietary adherence, disease activity), urine collection (dietary adherence - urine-gluten immunogenic peptide detection), blood collection (hemorheological, hemostatic, and immunological tests complemented with routine laboratory panel)
Key inclusion and exclusion criteria	Inclusion criteria: adult patients (≥ 18 years of age) suffering from newly diagnosed or treated celiac disease (by ESPGHAN and ACG guidelines), or from inflammatory bowel disease (by ECCO guidelines), and non-celiac, non-IBD subjects
	Exclusion criteria: chronic diseases (chronic kidney diseases, liver cirrhosis, heart failure, active malignant diseases), acute diseases within 2 weeks of inclusion
Study type	Observational
Date of first enrolment	30/5/2018
Target sample size	First phase: 50 celiac and 50 IBD patients plus control (1–3 for each patient). Second phase: target number is determined by power calculation.
Recruitment status	Ongoing
Primary outcome(s)	Hemorheological test results
Key secondary outcomes	Hemostatic test results

186

187 **Trial organization and steering committee**

188 The Centre for Translational Medicine at the University of Pécs, which was established to
 189 advance medical research in gastroenterology, is the coordinator and designer of the
 190 HERMES study. The centre is experienced in running investigator-initiated clinical trials.[51]
 191 A steering committee will be set up to supervise the entire study process. The Principal
 192 Investigator (JB) and the Trial Coordinator (ZS) are responsible for organizing patient
 193 recruitment, data collection, sample collection, shipping, and storage, biochemical analysis,
 194 and the publication of study results.

195

196 **Population and eligibility**

197 We will include CeD patients, IBD patients, and non-CeD, non-IBD control subjects.

198 Eligibility criteria will be as follows:

199 a. Inclusion criteria (applies to all subjects)

200 ➤ Blood collection must be indicated with medical conditions

201 ➤ Signed informed consent

202 b. Inclusion criteria (applies to specific cohorts of patients)

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3 203 ➤ CeD patients: newly diagnosed or followed patients (with or without adhering to
4 204 GFD) aged ≥ 18 years; the establishment of a diagnosis should meet the current
5 205 guidelines (ESPHGAN, ACG).[5, 52]
6
7 206 ➤ IBD patients: newly diagnosed or followed-up patients (with active or remitting
8 207 disease) aged ≥ 18 years (not following GFD); the establishment of a diagnosis
9 208 should meet the current guidelines (ECCO).[53, 54]
10
11 209 ➤ Non-CeD, non-IBD control subjects: individuals aged ≥ 18 years (not following
12 210 GFD) in whom CeD and IBD can be excluded according to the recent
13 211 guidelines. [5, 52-54]
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17 212 c. Exclusion criteria (applies to all subjects)
18
19 213 ➤ Chronic conditions:
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21 214 ➤ Estimated glomerular filtration rate calculated with CKD-EPI formula is
22 215 $< 60 \text{ ml/min/1.73m}^2$ (CKD3 or more severe kidney failure)
23
24 216 ➤ Liver cirrhosis in Child–Pugh B–C
25 217 ➤ Heart failure (NYHA III–IV)
26 218 ➤ Active malignant diseases
27
28 219 ➤ Any acute diseases or invasive procedures within two weeks of recruitment (e.g.,
29 220 systemic infection, surgery, or major trauma)
30
31 221 ➤ Pregnancy
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33 222 ➤ Patients unable to understand the essentials of the informed consent
34

223 **Flow and timing**

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37 224 All subjects at our academic hospital for a planned check-up or referred to the center for
38 225 diagnostic purposes will be recruited consecutively. The place of recruitment will be the
39 226 Division of Gastroenterology, First Department of Medicine, University of Pécs Medical
40 227 School. This tertiary centre provides professional gastroenterological care for about 300,000
41 228 inhabitants in Baranya County, Hungary.

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45 229 Recruitment of the study population will be managed in two phases (see ‘Target number of
46 230 patient’ section), with the expected recruiting period being between May 2018 and May 2019
47 231 (covering one year). Table 2 shows the timeline of the study. Patients will be provided with an
48 232 information sheet and must provide written consent before sampling. Informed consent will
49 233 be obtained by personnel with a medical degree. Participants may withdraw from the study for
50 234 any reason at any time. Consent forms and other related documents will be accessible at
51 235 <https://tm-centre.org>.

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237 Table 2. Schedule for the study

TIME POINT	Study period				
	Enrolment	Allocation	Post-allocation		
	-1 hour	0	+1 hour	+1.5 hour	+2 hour
ENROLMENT:					
Eligibility screen	x				
Informed consent	x				
Allocation		x			
INTERVENTION:					
Interview and questionnaire			x		
Urine collection				x	
Blood collection				x	
ASSESSMENT					
Symptom scores and disease activity			x		
Thrombophilia questionnaire			x		
Dietary adherence			x		
Blood analysis [#]					x
Urine analysis [#]					=>*

238

239 * samples will be deep frozen until all participants have been recruited

240 [#]after analysis, blood and urine residues will be stored in the biobank

241

242 Patients will be monitored by our professional data management team throughout the entire
 243 process of data and biological sample collection to ensure perfect adherence to protocol.
 244 Written feedback will be provided to patients on the results of the laboratory tests and dietary
 245 evaluation. If findings indicate, patients will be referred to their general practitioners or a
 246 specialist for further investigation and management.

247

248 **Measurements**

249 All samples will be collected and questionnaires will be administered within two hours
 250 after allocation. Actions for each group are defined and listed in Table 3.

251

252 Table 3. Actions within study

	CeD patients	IBD patients	Control subjects
Thrombophilia questionnaire	+	+	+
GSRS	+	-	+
Dietary interview and GFD adherence tests	+	-	+
Mayo Score/CDAI	-	+	+

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Urine GIP detection	+	-	+
Laboratory measures			
routine parameters	+	+	+
hemorheology	+	+	+
hemostasis	+	+	+
immunological indicators	+	+	+

253

254 CeD: celiac disease; CDAI: Crohn's Disease Activity Index; GFD: gluten-free diet; GIP:
255 gluten-immunogenic peptides; GSRS: Gastrointestinal Symptoms Rating Scale; IBD:
256 inflammatory bowel disease

257

258 Detailed history (including medications for preceding three months) and risk factors of
259 venous and arterial thrombotic events will be covered with a 15-minute thrombophilia
260 questionnaire (administered by a person with a medical degree).

261 The Gastrointestinal Symptoms Rating Scale is a tool designed to assess the severity of
262 gastrointestinal symptoms on a scale of 1 to 7 (administered by a person with a medical
263 degree).[55]

264 Disease activity in IBD will be estimated with either the (modified) Mayo Score[56] or
265 Crohn's Disease Activity Index[57] in patients with ulcerative colitis and Crohn's disease,
266 respectively, while tissue transglutaminase (tTG) levels will be used to measure the activity of
267 CeD. (Scores will be determined by the gastroenterologist enrolling the patient.)

268 Dietary adherence of CeD patients will be estimated through (1) a dietary interview
269 conducted by a trained dietitian on a scale of 1 to 10, (2) self-reporting,[58] (3) a test
270 measuring knowledge of gluten-free foods, (4) urine GIP detection (details in the text), and
271 (5) celiac-specific serology (tTG and endomysium antibody levels (EMA)).

272 All laboratory tests will be performed in the same laboratory (University of Pécs, Hungary)
273 from venous blood. Blood samples will be collected in plastic tubes prospectively (2 x BD
274 Vacutainer 10.0 ml (red), 2 x BD Vacutainer 6.0 ml (purple), 1 x BD Vacutainer 3.0 ml
275 (pink), 1 x BD Vacutainer 2.7 ml (blue), and 1 x BD Seditainer 5.0 ml (black) for a total of
276 42.7 ml blood from each patient (BD, USA)).

277 We will measure:

- 278 • routine laboratory parameters: bilirubin, urea, creatinine, cholesterol (total, high-
279 density and low-density lipoproteins), triglyceride, aspartate, aspartate
280 aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl
281 transferase, total protein, albumin, immunoglobulins, C-reactive protein, vitamin B₁₂,
282 homocysteine, blood counts, and erythrocyte sedimentation

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3 283 • immunological indicators: antiphospholipid antibodies (lupus anticoagulant,
4 284 cardiolipin IgG/A/M, B2-glycoprotein-I IgG/A/M, prothrombin IgG/A/M) and celiac-
5 285 specific antibodies (tTG IgA/G, EMA IgA).
6
7
8 286 • hemostatic parameters: prothrombin, thrombin time, activated partial thromboplastin
9 287 time, fibrinogen, antithrombin activity, protein C activity, and protein S activity
10
11 288 • hemorheological parameters: erythrocyte aggregation by Myrenne aggregometer
12 289 (model MA-1, Myrenne GmbH, Roetgen, Germany) and Laser-assisted Optical
13 290 Rotational Cell Analyzer (LORCA, R&R Mechatronics, Hoorn, The Netherlands);
14 291 erythrocyte deformability with laser-diffraction ektacytometry with a LORCA; and
15 292 viscosity of whole blood and plasma by Brookfield DV-III Ultra LV Programmable
16 293 rotational viscometer (Brookfield Engineering Labs; Middleboro, Mass., USA).

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21 294 Strict adherence will be kept during the hemorheological tests to the guidelines proposed
22 295 by the International Expert Panel for Standardization of Hemorheological Methods.[59] The
23 296 fact that equipment for hemorheological measurements is not available in other centres in
24 297 Hungary and that blood samples must be processed within two hours of sampling without
25 298 freezing restricted our expansion of this project to a multicentre study.

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29 299 An extra tube will be collected and stored for further hemostatic measurements (e.g.,
30 300 clotting factors) if any abnormality of parameters measured is detected.

31
32 301 Midstream urine (at least 100 ml) will be collected in sterile urine sample containers.
33 302 Samples will be stored at 4°C until transfer to the Biobank at the Institute for Translational
34 303 Medicine, University of Pécs Medical School, on the day of sampling, where samples will be
35 304 deep frozen at -80°C. After preparation, urine GIP detection will be performed with Biomedal
36 305 (Spain) products.

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41 307 **Outcomes**

42 308 1. Primary

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45 309 • Hemorheological test results (erythrocyte aggregation and deformability, whole
46 310 blood and plasma viscosity).

47 311 2. Secondary

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50 312 • Hemostatic test results (antithrombin, protein C, protein S).
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52 314 **Target number of patients**

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315 This is a two-phase study. In the first phase, we will enrol 50 CeD and 50 IBD patients
 316 with 50 age- and sex-matched control subjects; the case-control ratio will be 1:1:1,
 317 respectively. Then, an interim analysis will be performed to calculate the power for the
 318 analyses of the outcomes. If the power exceeds 80%, recruitment will be considered
 319 completed; otherwise, recruitment will continue until the desired power is reached.

320

321 **Patient and Public Involvement**

322 Before starting recruitment, randomly selected CeD and IBD patients reviewed the
 323 questionnaires and the information sheet designed to share details of the study for participants
 324 to facilitate better understanding.

325

326 **Blinding**

327 Blinding of personnel included in the study is presented in Table 4.

328

329 Table 4. Blinding of personnel included in the study

	Physician enrolling patient	Physician administering questionnaires	Dietitian	Laboratory personnel
Disease activity	N/A	Blinded	Blinded	Blinded
Questionnaires	Blinded*	N/A	Blinded	Blinded
Dietary interview	Blinded*	Blinded	N/A [#]	Blinded
Laboratory measures	Blinded*	Blinded	Blinded	N/A

330

331 N/A: not applicable.

332 *The treating physician will immediately access data for safety reasons and act accordingly.

333 Patients will be informed of the laboratory results in a letter.

334 [#]Dietary education will be provided based on dietary adherence.

335

336 **Data management**

337 A subject identification number will be provided consecutively to every patient after
 338 inclusion. Subject identification numbers with sensitive data on patients (including the name,
 339 insurance number, and date of enrolment) will be stored in a locked file separately from other
 340 data. De-identified data will be added to the source documentation stored in locked cabinets.
 341 Source documentation will be entered in an electronic case report file (e-CRF). The Principal
 342 Investigators will ensure that the data in an e-CRF are accurate, complete, and legible (range
 343 checks for data values). E-CRFs will be stored on a secured server at the Institute for
 344 Translational Medicine, University of Pécs Medical School. Access to data will be restricted
 345 through a password system to personnel involved in data management. A three-level data

1
2
3 346 check will be continuously performed, and final data will be finally approved by the Principal
4 347 Investigator to ensure data quality.

5
6 348 To ensure precise data collection, administrative and medical staff members will be invited
7 349 to participate in training sessions to familiarize them with the study requirements,
8 350 standardized data recording, and biological specimen collection.

9
10
11 351 The de-identified dataset will be delivered for the purpose of sharing on request.
12 352

13 14 353 **Statistical Analysis**

15 354 First, descriptive statistics will entail a graphical presentation of data. Continuous variables
16 355 will be reported as a central tendency with a measure of dispersion, while categorical
17 356 variables will be reported as absolute and relative frequencies. Then, data will be analyzed
18 357 with Student's tests, methods of Variance Analysis, and regression models if data are
19 358 normally distributed; otherwise, non-parametric tests will be introduced. Chi-square or
20 359 Fisher's tests will be used to analyze categorical variables. Multivariate analysis will be used
21 360 to explore the association between thrombotic risk factors and primary outcomes. A
22 361 probability of less than .05 indicates a statistically significant difference between groups.

23
24 362 Only patients with a full dataset in their hemorheological and hemostatic profile will be
25 363 included in the analysis. The following comparisons will be done: CeD vs. control, tTG+ CeD
26 364 vs. tTG- CeD, IBD vs. control, active IBD vs. remitting IBD.

27 365 An interim analysis is planned after recruiting the target number of the first phase to
28 366 calculate power. Audits are not necessary due to the case-control design.
29 367

30 31 368 **Biobank and accessory research**

32 369 After laboratory analysis, urine and blood (whole blood and plasma, at least 1 ml each)
33 370 residues will be stored in the Institute for Translational Medicine Biobank at -80°C for future
34 371 studies (for at least five years). Additional samples will not be taken for storage purposes.
35 372 Containers will be labelled with the subject identification number, and samples will be
36 373 completely de-identified.

37
38 374 CeD patients will be offered an opportunity to participate in the "Monitoring the
39 375 prevalence, symptoms, complications, and family history of celiac disease and the effect of a
40 376 gluten-free diet – Celiac registry" research project (approved by the Scientific and Research
41 377 Ethics Committee of the Medical Research Council, Ref No 45098-2/2016/EKU).
42 378

43 44 379 **Protocol amendments and disseminating policy**

1
2
3 380 This protocol is the first version completed on 30 May 2018. If required, the online version
4 381 will be updated in the ISRCTN registry. Major modifications should be permitted by the
5
6 382 Regional and Local Research Ethics Committee.

7 383 The trial status is ongoing; recruitment began on 1 May 2018. The expected date of
8 384 completion is 31 May 2019.

9
10
11 385

12 386 **ETHICS AND DISSEMINATION**

13
14 387 The study was approved by the Regional and Local Research Ethics Committee,
15 388 University of Pécs (Ref No 6917). Publication in a high-impact peer-reviewed journal is
16
17 389 planned. We will adhere to authorship criteria for manuscripts submitted for publication set
18
19 390 by the International Committee of Medical Journal Editors.

20
21 391

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53 524 **AUTHORS' CONTRIBUTIONS**

1
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3 525 JB is the Principal Investigator. ZS is the Trial Coordinator. ZS, PH, JB, ÁV, and KT
4 526 conceptualized the study, drafted, and revised this manuscript. NF and EB planned and
5 527 drafted the statistical analysis. PS, JB, and ÁV provided us with special expertise in the
6 528 management of celiac disease and inflammatory bowel patients. BC and PK are performing
7 529 the hemorheological measurements and interpreting the results. AH, ÁN, TB, and MTF
8 530 provided us with special expertise in hemostatic and immunological measurements. BK is
9 531 contributing significantly to the biochemical analyses. IV planned and is carrying out the
10 532 dietary assessment of the celiac patients. KM, AS, ZS, and PH are responsible for data
11 533 management, administrative coordination, and biological sampling; they drafted and revised
12 534 the manuscript. All the authors have read and approved the final manuscript.
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545

546 **COMPETING INTEREST STATEMENT**

547 Nothing to declare.

BMJ Open

Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol

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Keywords :	Inflammatory bowel disease < GASTROENTEROLOGY, Coeliac disease < GASTROENTEROLOGY, thrombosis, hemorheology

SCHOLARONE™
Manuscripts

1 Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease
2 in comparison with non-celiac, non-IBD subjects (HERMES): A case-control study protocol

3
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35 **Abstract**

37 **Introduction**

38 Hemorheological and hemostatic changes predispose to the development of arterial and
39 venous thrombotic events; however, limited information is available on the status of these
40 changes in celiac disease (CeD) and inflammatory bowel disease (IBD). In this study, we aim
41 to describe the hemorheological and hemostatic profiles of CeD and IBD patients in a
42 Hungarian cohort of patients to investigate whether any alterations contribute to elevated
43 thrombotic risk.

44 **Methods and analysis**

45 This is a case-control study involving newly diagnosed and followed CeD and IBD patients
46 with age- and sex-matched non-CeD, non-IBD subjects with an allocation ratio of 1:1:1.

47 After informed consent is obtained, a detailed medical history will be collected, including
48 venous and arterial thrombotic risk factors and medications. Symptoms in CeD patients will be
49 assessed with the Gastrointestinal Symptoms Rating Scale, and disease activity in IBD patients
50 will be determined by disease-specific scores. Dietary adherence will be assessed among CeD
51 patients with a thorough interview together with a measurement of self-reported adherence,
52 dietary knowledge, and urine analysis (detection of gluten immunogenic peptides). In addition
53 to routine laboratory parameters, hemorheological (i.e., erythrocyte deformability and
54 aggregation, viscosity of whole blood and plasma) and hemostatic parameters (e.g., protein C,
55 protein S, and antithrombin) with immunological indicators (i.e., celiac-specific serology and
56 antiphospholipid antibodies) will be measured from venous blood for every participant.

57 Primary and secondary outcomes will be hemorheological and hemostatic parameters,
58 respectively. Univariate and multivariate statistics will be used to compare CeD and IBD
59 patients to control subjects. Subgroup analysis will be performed by disease type in IBD,
60 (Crohn's disease and ulcerose colitis), dietary adherence in CeD, and disease activity in IBD
61 and CeD.

62 **Ethics and dissemination**

63 The study was approved by the Regional and Local Research Ethics Committee, University
64 of Pécs (Ref No 6917). Findings will be disseminated at research conferences and in peer-
65 reviewed journals.

66 **Trial registration**

67 ISRCTN49677481.

68

69 **Key words:** celiac disease, inflammatory bowel disease, hemorheology, thrombosis

71 **Strengths and limitations of this study**

- 72 • Immune-mediated bowel diseases are associated with an increased risk of arterial and
73 venous thrombosis, but specific hemorheological and hemostatic alterations are
74 understudied in celiac disease and incomplete in inflammatory bowel disease.
- 75 • This case-control study prospectively recruits newly diagnosed and followed-up cases
76 of celiac disease and inflammatory bowel disease with age- and sex-matched controls
77 (the allocation ratio will be 1:1:1, respectively) to investigate clinical and laboratory
78 alterations predisposing to thrombosis.
- 79 • Laboratory tests include the measurement of hemorheological (i.e., erythrocyte
80 aggregation and deformability, plasma and whole blood viscosity), hemostatic
81 parameters (e.g., levels of fibrinogen, prothrombin time, protein C, protein S, and
82 antithrombin), and immunological indicators (e.g., celiac-specific serology and
83 antiphospholipid antibodies).
- 84 • Patients will be divided by disease activity into active and inactive.
- 85 • Results should be interpreted with caution due to the single-centre nature and case-
86 control design of the study.

88 **INTRODUCTION**

89 Immune-mediated disorders may affect 5–7% of the population.[1] These disorders
90 frequently share pathways in pathogenesis as well as organ manifestations. Celiac disease
91 (CeD) and inflammatory bowel disease (IBD) are systemic disorders, primarily affecting the
92 intestines.[2] They impose a significant burden of complications and concomitant diseases on
93 patients during the disease course.

94 CeD is a chronic, immune-mediated disorder, which develops upon gluten ingestion in
95 genetically susceptible individuals.[3] Global prevalence of CeD is around 1% with
96 geographical differences ranging from 0.14% up to 5.7%.[3] The clinical presentation can be
97 divided into classic, non-classic, and asymptomatic forms.[4] Diagnosing asymptomatic and
98 atypical cases is challenging but important, because the disease course of these cases may be
99 alike.[5]

100 IBD – clinically classified as Crohn’s disease or ulcerative colitis – is a chronic, relapsing
101 disorder, which develops as a result of the interaction between environmental and genetic

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3 102 factors, leading to immunological responses and inflammation in the gastrointestinal tract.[6]
4
5 103 IBD is a less frequent entity than CeD: the increasing prevalence of ulcerative colitis and
6
7 104 Crohn's disease may reach 0.5% and 0.3% in Europe, respectively.[7, 8]

8
9 105 Immune-mediated disorders may be associated with hemorheological[9-11] and hemostatic
10
11 106 changes[12-14], thereby contributing to an increased risk of thrombotic events.[15] This
12
13 107 increased risk is manifested in CeD[16] and IBD.[17] Mechanisms of thrombophilia in
14
15 108 immune-mediated disorders are complex, and acquired factors seem important.[18] An altered
16
17 109 hemorheological profile as well as the altered levels or function of pro- and anticoagulant
18
19 110 proteins, altered activity of clotting factors contribute to the development of arterial and venous
20
21 111 thrombotic events.[19-23]

22
23 112 Clinical presentation of CeD-associated hypercoagulability includes a wide variety of
24
25 113 thrombosis at venous sites, pulmonary embolism, atheroembolism (stroke), and obstetric
26
27 114 complications.[24, 25] A single retrospective publication examined hemostatic alterations in a
28
29 115 small cohort of patients: sporadic cases of protein C and protein S deficiency (due to vitamin K
30
31 116 malabsorption), hyperhomocysteinemia, and antiphospholipid antibodies were identified.[26]
32
33 117 No studies have assessed the hemorheological changes in CeD. The multifactorial etiology of
34
35 118 thrombosis may embrace the interplay of malabsorption (vitamin and mineral deficiencies, e.g.,
36
37 119 vitamin B₁₂ and K deficiency), thrombophilic autoantibodies (anti-tissue transglutaminase
38
39 120 (tTG) and antiphospholipid antibodies), hyperhomocysteinemia, endothelial dysfunction,
40
41 121 accelerated atherosclerosis, thrombocytosis and thrombocyte dysfunction, hyperviscosity, and
42
43 122 genetics.[24, 26-31] Immune-mediated comorbidities ('autoimmune traits'), such as
44
45 123 antiphospholipid syndrome, may contribute to the elevated thrombotic risk as well.[29] In
46
47 124 addition, ingestion of trace amounts of gluten may maintain a continuous pro-inflammatory
48
49 125 response.[32]

50
51 126 IBD is associated with venous thrombosis and pulmonary embolism as well as with the
52
53 127 cardiovascular consequences of atherosclerosis, i.e., stroke and myocardial infarction.[33, 34]
54
55 128 A significant decline in anticoagulant mechanism is well-established and there are sporadic
56
57 129 reports on activity-dependent prothrombotic hemorheological changes.[35-38] However, while
58
59 130 individual studies have focused on single outcomes of laboratory parameters, none of them have
60
61 131 assessed the complete hemorheological profile of patients.[39-42] Other risk factors include
62
63 132 immobilization, surgical interventions, glucocorticoid therapy, vitamin deficiencies,
64
65 133 hyperhomocysteinemia, and chronic inflammation alone or in conjunction with the factors
66
67 134 above.[43, 44] In the case of IBD, disease activity may be a crucial determinant of thrombotic
68
69 135 risk.[34]

136

137 **Scope and objectives**

138 No studies have assessed hemorheological and hemostatic parameters within a study to
 139 provide an overall view of thrombotic risk. Since our knowledge of hemorheological and
 140 hemostatic changes is limited in CeD and IBD, this study aims to carry out a comprehensive
 141 evaluation of venous and arterial prothrombotic alterations in these pro-inflammatory diseases
 142 in a Hungarian cohort of patients.

143 1. Primary objective

- 144 • to identify a link between prothrombotic hemorheological and hemostatic alterations
 145 and two common immune-mediated diseases (CeD and IBD)

146 2. Secondary objective

- 147 • to investigate the effect of disease activity on the hemorheological and hemostatic
 148 profiles of CeD and IBD patients
- 149 • to find an association between the dietary adherence of CeD patients and the
 150 hemorheological and hemostatic alterations
- 151 • to assess the modifying effect of immunosuppressant drugs in IBD on the
 152 hemorheological and hemostatic profiles

153 **METHODS AND ANALYSIS**

155 **Design**

156 This is a case-control study with prospective recruitment of CeD and IBD patients with non-
 157 CeD, non-IBD control subjects. The study does not change the routine management of subjects
 158 included (for the World Health Organization checklist, see Table 1). The study protocol was
 159 planned in accordance with the SPIRIT 2013 Statement.[45]

161 Table 1. World Health Organization checklist

48 Data category	49 Information
50 Primary registry and trial 51 identifying number	ISRCTN49677481
52 Date of registration in primary 53 registry	05/03/2018
54 Secondary identifying numbers	None
55 Source(s) of monetary or material 56 support	57 University of Pécs Medical School; Momentum Grant from the 58 Hungarian Academy of Sciences (LP2014-10/2014); Highly Cited 59 Publication Grant (KH 125678) from the National Research 60 Development and Innovation Office; GINOP 2.3.2-15-2016-00048 Stay Alive, EFOP 3.6.2-16-2017-00006 Live Longer, and EFOP-

	3.6.3-VEKOP-16-2017-00009; Translational Medicine Foundation; and New National Excellence Programme, Ministry of Human Capacities (ÚNKP-17-3-II, ÚNKP-18-3-I).
Primary sponsor	None
Secondary sponsor(s)	None
Contact for public queries	Zsolt Szakács, MD, szakacs.zsolt@pte.hu
Contact for scientific queries	Judit Bajor, MD, bajor.judit@pte.hu
Public title	Investigation of hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with healthy subjects: A case-control study (HERMES)
Scientific title	Hemorheological and hemostatic alterations in celiac disease and inflammatory bowel disease in comparison with non-celiac, non-IBD subjects: A case-control study (HERMES)
Countries of recruitment	Hungary
Health condition(s) or problem(s) studied	Celiac disease and inflammatory bowel disease
Intervention(s)	Questionnaires (thrombophilia, dietary adherence, disease activity), urine collection (dietary adherence - urine-gluten immunogenic peptide detection), blood collection (hemorheological, hemostatic, and immunological tests complemented with routine laboratory panel)
Key inclusion and exclusion criteria	Inclusion criteria: adult patients (≥ 18 years of age) suffering from biopsy-confirmed newly diagnosed or treated celiac disease (by ESPHGAN, ACG, WGO guidelines), or from inflammatory bowel disease (by ECCO guidelines), and non-celiac, non-IBD subjects Exclusion criteria: chronic diseases (chronic kidney diseases, liver cirrhosis, heart failure, active malignant diseases), acute diseases within 2 weeks of inclusion, pregnancy, thrombotic events within 1 year, systematic lupus erythematosus, and use of oral anticoagulants or antiplatelet therapy
Study type	Observational
Date of first enrolment	30/5/2018
Target sample size	First phase: 50 celiac and 50 IBD patients plus control (1–3 for each patient). Second phase: target number is determined by power calculation.
Recruitment status	Ongoing
Primary outcome(s)	Hemorheological test results
Key secondary outcomes	Hemostatic test results

162

163 Trial organization and steering committee

164 The Centre for Translational Medicine at the University of Pécs, which was established to
 165 advance medical research in gastroenterology, is the coordinator and designer of the HERMES
 166 study. The centre is experienced in running investigator-initiated clinical trials.[46] A steering
 167 committee will be set up to supervise the entire study process. The Principal Investigator (JB)
 168 and the Trial Coordinator (ZS) are responsible for organizing patient recruitment, data

1
2
3 169 collection, sample collection, shipping, and storage, biochemical analysis, and the publication
4 of study results.
5 170
6
7 171

8 172 **Population and eligibility**

9
10 173 We will include CeD patients, IBD patients, and non-CeD, non-IBD control subjects.
11 Eligibility criteria will be as follows:
12 174

13 175 a. Inclusion criteria (applies to all subjects)

- 14 176 ➤ Blood collection must be indicated with medical conditions
- 15 177 ➤ Signed informed consent

16 178 b. Inclusion criteria (applies to specific cohorts of patients)

- 17 179 ➤ CeD patients: biopsy-confirmed newly diagnosed or followed patients (with or
18 without adhering to a gluten-free diet) aged ≥ 18 years; the establishment of a
19 diagnosis should meet the current guidelines (ESPHGAN, ACG).[3, 47, 48]
- 20 182 ➤ IBD patients: newly diagnosed or followed-up patients (with active or remitting
21 disease) aged ≥ 18 years (not following a gluten-free diet); the establishment of a
22 diagnosis should meet the current guidelines (ECCO).[49, 50]
- 23 185 ➤ Non-CeD, non-IBD control subjects: individuals aged ≥ 18 years (not following a
24 gluten-free diet) in whom CeD and IBD can be excluded according to the recent
25 guidelines. [3, 47-50]

26 188 c. Exclusion criteria (applies to all subjects)

- 27 189 ➤ Chronic conditions:
 - 28 190 ➤ Estimated glomerular filtration rate calculated with CKD-EPI formula is
 - 29 191 $< 60 \text{ ml/min/1.73m}^2$ (CKD3 or more severe kidney failure)
 - 30 192 ➤ Liver cirrhosis in Child–Pugh B–C
 - 31 193 ➤ Heart failure (NYHA III–IV)
 - 32 194 ➤ Active malignant diseases
- 33 195 ➤ Any acute diseases or invasive procedures within two weeks of recruitment (e.g.,
34 systemic infection, surgery, or major trauma)
- 35 197 ➤ Thrombotic events within 1 year of recruitment
- 36 198 ➤ Ongoing oral anticoagulant therapy (vitamin K antagonists) and/or antiplatelet
37 drugs
- 38 200 ➤ Confirmed systemic lupus erythematosus
- 39 201 ➤ Pregnancy
- 40 202 ➤ Patients unable to understand the essentials of the informed consent

203 Flow and timing

204 All subjects at our academic hospital for a planned check-up or referred to the center for
 205 diagnostic purposes will be recruited consecutively. The place of recruitment will be the
 206 Division of Gastroenterology, First Department of Medicine, University of Pécs Medical
 207 School. This tertiary centre provides professional gastroenterological care for about 300,000
 208 inhabitants in Baranya County, Hungary.

209 Recruitment of the study population will be managed in two phases (see ‘Target number of
 210 patient’ section), with the expected recruiting period being between May 2018 and May 2019
 211 (covering one year). Table 2 shows the timeline of the study. Patients will be provided with an
 212 information sheet and must provide written consent before sampling. Informed consent will be
 213 obtained by personnel with a medical degree. Participants may withdraw from the study for any
 214 reason at any time. Consent forms and other related documents will be accessible at [https://tm-](https://tm-centre.org)
 215 [centre.org](https://tm-centre.org).

217 Table 2. Schedule for the study

TIME POINT	Study period				
	Enrolment	Allocation	Post-allocation		
	-1 hour	0	+1 hour	+1.5 hour	+2 hour
ENROLMENT:					
Eligibility screen	x				
Informed consent	x				
Allocation		x			
INTERVENTION:					
Interview and questionnaire			x		
Urine collection				x	
Blood collection				x	
ASSESSMENT					
Symptom scores and disease activity			x		
Thrombophilia questionnaire			x		
Dietary adherence			x		
Blood analysis [#]					x
Urine analysis [#]					=>*

218

219 * samples will be deep frozen until all participants have been recruited

220 [#]after analysis, blood and urine residues will be stored in the biobank

221

222 Patients will be monitored by our professional data management team throughout the entire
 223 process of data and biological sample collection to ensure perfect adherence to protocol. Written
 224 feedback will be provided to patients on the results of the laboratory tests and dietary evaluation.
 225 If findings indicate, patients will be referred to their general practitioners or a specialist for
 226 further investigation and management.

227

228 **Measurements**

229 All samples will be collected and questionnaires will be administered within two hours after
 230 allocation. Actions for each group are defined and listed in Table 3.

231

232 Table 3. Actions within study

	CeD patients	IBD patients	Control subjects
Thrombophilia questionnaire	+	+	+
GSRs	+	-	+
Dietary interview and GFD adherence tests	+	-	+
Mayo Score/CDAI	-	+	+
Urine GIP detection	+	-	+
Laboratory measures			
routine parameters	+	+	+
hemorheology	+	+	+
hemostasis	+	+	+
immunological indicators	+	+	+

233

234 CeD: celiac disease; CDAI: Crohn's Disease Activity Index; GFD: gluten-free diet; GIP:
 235 gluten-immunogenic peptides; GSRs: Gastrointestinal Symptoms Rating Scale; IBD:
 236 inflammatory bowel disease

237

238 Detailed history (including medications for preceding three months) and risk factors of
 239 venous and arterial thrombotic events will be covered with a 15-minute thrombophilia
 240 questionnaire (administered by a person with a medical degree).

241 The Gastrointestinal Symptoms Rating Scale is a tool designed to assess the severity of
 242 gastrointestinal symptoms on a scale of 1 to 7 (administered by a person with a medical
 243 degree).[51]

244 Disease activity in IBD will be estimated with either the (modified) Mayo Score[52] or
 245 Crohn's Disease Activity Index[53] in patients with ulcerative colitis and Crohn's disease,
 246 respectively, while tissue transglutaminase (tTG) levels will be used to measure the activity of
 247 CeD. (Scores will be determined by the gastroenterologist enrolling the patient.)

1
2
3 248 Dietary adherence of CeD patients will be estimated through (1) a dietary interview
4
5 249 conducted by a trained dietitian on a scale of 1 to 10, (2) self-reporting,[54] (3) a test measuring
6
7 250 knowledge of gluten-free foods, (4) urine GIP detection (details in the text), and (5) celiac-
8
9 251 specific serology (tTG and endomysium antibody levels (EMA)).[55] Patients will be divided
10
11 252 into those with good and poor dietary adherence based on the complex assessment of the above-
12
13 253 mentioned data.

14 254 All laboratory tests will be performed in the same laboratory (University of Pécs, Hungary)
15
16 255 from venous blood. Blood samples will be collected in plastic tubes prospectively (2 x BD
17
18 256 Vacutainer 10.0 ml (red), 2 x BD Vacutainer 6.0 ml (purple), 1 x BD Vacutainer 3.0 ml (pink),
19
20 257 1 x BD Vacutainer 2.7 ml (blue), and 1 x BD Seditainer 5.0 ml (black) for a total of 42.7 ml
21
22 258 blood from each patient (BD, USA)).

23 259 We will measure:

- 24 260 • routine laboratory parameters: bilirubin, urea, creatinine, cholesterol (total, high-density
25
26 261 and low-density lipoproteins), triglyceride, aspartate, aspartate aminotransferase,
27
28 262 alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total
29
30 263 protein, albumin, immunoglobulins, C-reactive protein, vitamin B₁₂, folic acid,
31
32 264 homocysteine, blood counts, and erythrocyte sedimentation
- 33 265 • immunological indicators: antiphospholipid antibodies (lupus anticoagulant, cardiolipin
34
35 266 IgG/A/M, B2-glycoprotein-I IgG/A/M, prothrombin IgG/A/M) and celiac-specific
36
37 267 antibodies (tTG IgA/G, EMA IgA).
- 38 268 • hemostatic parameters: prothrombin, thrombin time, activated partial thromboplastin
39
40 269 time, fibrinogen, antithrombin activity, protein C activity, and protein S activity
- 41
42 270 • hemorheological parameters: erythrocyte aggregation by Myrenne aggregometer
43
44 271 (model MA-1, Myrenne GmbH, Roetgen, Germany) and Laser-assisted Optical
45
46 272 Rotational Cell Analyzer (LORCA, R&R Mechatronics, Hoorn, The Netherlands);
47
48 273 erythrocyte deformability with laser-diffraction ektacytometry with a LORCA; and
49
50 274 viscosity of whole blood and plasma by Brookfield DV-III Ultra LV Programmable
51
52 275 rotational viscometer (Brookfield Engineering Labs; Middleboro, Mass., USA). The
53
54 276 Case Report Form providing data about the measurements is presented in
55
56 277 Supplementary material.

57 278 Strict adherence will be kept during the hemorheological tests to the guidelines proposed by
58
59 279 the International Expert Panel for Standardization of Hemorheological Methods.[56] The fact
60
280 that equipment for hemorheological measurements is not available in other centres in Hungary

281 and that blood samples must be processed within two hours of sampling without freezing
282 restricted our expansion of this project to a multicentre study.

283 An extra tube will be collected and stored for further hemostatic measurements (e.g., clotting
284 factors) if any abnormality of parameters measured is detected.

285 Midstream urine (at least 100 ml) will be collected in sterile urine sample containers.
286 Samples will be stored at 4°C until transfer to the Biobank at the Institute for Translational
287 Medicine, University of Pécs Medical School, on the day of sampling, where samples will be
288 deep frozen at -80°C. After preparation, urine GIP detection will be performed with Biomedal
289 (Spain) products.

290

291 **Outcomes**

292 1. Primary

- 293 • Hemorheological test results (erythrocyte aggregation and deformability, whole
294 blood and plasma viscosity).

295 2. Secondary

- 296 • Hemostatic test results (antithrombin, protein C, protein S), folic acid, and
297 homocysteine levels.

298

299 **Target number of patients**

300 This is a two-phase study. In the first phase, we will enrol 50 CeD and 50 IBD patients with
301 50 age- and sex-matched control subjects; the case-control ratio will be 1:1:1, respectively.
302 Then, an interim analysis will be performed to calculate the power for the analyses of the
303 outcomes. If the power exceeds 80%, recruitment will be considered completed; otherwise,
304 recruitment will continue until the desired power is reached.

305

306 **Patient and Public Involvement**

307 Before starting recruitment, randomly selected CeD and IBD patients reviewed the
308 questionnaires and the information sheet designed to share details of the study for participants
309 to facilitate better understanding.

310

311 **Blinding**

312 Blinding of personnel included in the study is presented in Table 4.

313

314 Table 4. Blinding of personnel included in the study

	Physician enrolling patient	Physician administering questionnaires	Dietitian	Laboratory personnel
Disease activity	N/A	Blinded	Blinded	Blinded
Questionnaires	Blinded*	N/A	Blinded	Blinded
Dietary interview	Blinded*	Blinded	N/A [#]	Blinded
Laboratory measures	Blinded*	Blinded	Blinded	N/A

315

316 N/A: not applicable.

317 *The treating physician will immediately access data for safety reasons and act accordingly.

318 Patients will be informed of the laboratory results in a letter.

319 [#]Dietary education will be provided based on dietary adherence.

320

321 **Data management**

322 A subject identification number will be provided consecutively to every patient after
 323 inclusion. Subject identification numbers with sensitive data on patients (including the name,
 324 insurance number, and date of enrolment) will be stored in a locked file separately from other
 325 data. De-identified data will be added to the source documentation stored in locked cabinets.
 326 Source documentation will be entered in an electronic case report file (e-CRF). The Principal
 327 Investigators will ensure that the data in an e-CRF are accurate, complete, and legible (range
 328 checks for data values). E-CRFs will be stored on a secured server at the Institute for
 329 Translational Medicine, University of Pécs Medical School. Access to data will be restricted
 330 through a password system to personnel involved in data management. A three-level data check
 331 will be continuously performed, and final data will be finally approved by the Principal
 332 Investigator to ensure data quality.

333 To ensure precise data collection, administrative and medical staff members will be invited
 334 to participate in training sessions to familiarize them with the study requirements, standardized
 335 data recording, and biological specimen collection.

336 The de-identified dataset will be delivered for the purpose of sharing on request.

337

338 **Statistical Analysis**

339 First, descriptive statistics will entail a graphical presentation of data. Continuous variables
 340 will be reported as a central tendency with a measure of dispersion, while categorical variables
 341 will be reported as absolute and relative frequencies. Then, data will be analyzed with Student's
 342 tests, methods of Variance Analysis, and regression models if data are normally distributed;
 343 otherwise, non-parametric tests will be introduced. Chi-square or Fisher's tests will be used to
 344 analyze categorical variables. Multivariate analysis will be used to take the most important
 345 thrombotic factors into account (e.g., the use of oral contraceptives and immunosuppressants,

1
2
3 346 previous thrombotic history, smoking, comorbidities). A probability of less than .05 indicates
4
5 347 a statistically significant difference between groups.

6 348 Only patients with a full dataset in their hemorheological and hemostatic profile will be
7
8 349 included in the analysis. The following comparisons will be done: CeD vs. control, tTG+ CeD
9
10 350 vs. tTG- CeD, CeD with good dietary adherence vs. CeD with poor dietary adherence, IBD vs.
11
12 351 control, active IBD vs. remitting IBD, and Crohn's disease vs. ulcerative colitis.

13 352 An interim analysis is planned after recruiting the target number of the first phase to calculate
14
15 353 power. Audits are not necessary due to the case-control design.

16
17 354

18 355 **Biobank and accessory research**

19
20 356 After laboratory analysis, urine and blood (whole blood and plasma, at least 1 ml each)
21
22 357 residues will be stored in the Institute for Translational Medicine Biobank at -80°C for future
23
24 358 studies (for at least five years). Additional samples will not be taken for storage purposes.
25
26 359 Containers will be labelled with the subject identification number, and samples will be
27
28 360 completely de-identified.

29 361 CeD patients will be offered an opportunity to participate in the "Monitoring the prevalence,
30
31 362 symptoms, complications, and family history of celiac disease and the effect of a gluten-free
32
33 363 diet – Celiac registry" research project (approved by the Scientific and Research Ethics
34
35 364 Committee of the Medical Research Council, Ref No 45098-2/2016/EKU).

36 365

37 366 **Protocol amendments and disseminating policy**

38
39 367 This protocol is the first version completed on 30 May 2018. If required, the online version
40
41 368 will be updated in the ISRCTN registry. Major modifications should be permitted by the
42
43 369 Regional and Local Research Ethics Committee.

44 370 The trial status is ongoing; recruitment began on 1 May 2018. The expected date of
45
46 371 completion is 31 May 2019.

47
48 372

49 373 **DISCUSSION**

50
51 374 Recent guidelines on CeD do not make any recommendations on how to prevent and manage
52
53 375 thrombotic events in CeD patients.[3, 47] Gluten-free diet, which is the only approved treatment
54
55 376 of the disease, may reduce or eliminate some thrombotic risk factors (e.g., consequences of
56
57 377 malabsorption and chronic inflammation) but it is uncertain whether the thrombotic risk
58
59 378 completely normalizes.[57] With respect to malabsorption, intestinal mucosa does not recover
60
379 in a high fraction of patients despite a long-term strict diet, particularly in those diagnosed in

1
2
3 380 the adulthood.[58] Whether CeD patients after a thrombotic event would benefit from a lifelong
4
5 381 anticoagulation therapy has remained unclear. The need for thromboprophylaxis under
6
7 382 prothrombotic circumstances, such as hospitalization, pregnancy, or immobilization should be
8
9 383 further investigated.

10 384 IBD guidelines recommend that thromboprophylaxis should be considered in all in- and
11
12 385 outpatients with an active disease.[59, 60] In addition to disease severity, the choice of
13
14 386 treatment influences the thrombotic risk as well.[17] A tool of personalized thrombotic risk
15
16 387 stratification including objective laboratory markers is awaited.

17 388 Our results can contribute to expanding our knowledge on the prothrombotic
18
19 389 pathophysiological alteration in CeD and IBD, thereby providing the basis for future research.

20 390

21 391 **ETHICS AND DISSEMINATION**

22
23
24 392 The study was approved by the Regional and Local Research Ethics Committee, University
25
26 393 of Pécs (Ref No 6917). Publication in a high-impact peer-reviewed journal is planned. We will
27
28 394 adhere to authorship criteria for manuscripts submitted for publication set by the International
29
30 395 Committee of Medical Journal Editors.

31 396

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11 532 **AUTHORS' CONTRIBUTIONS**

12 533 JB is the Principal Investigator. ZS is the Trial Coordinator. ZS, PH, JB, ÁV, and KT
13 534 conceptualized the study, drafted, and revised this manuscript. NF and EB planned and drafted
14 535 the statistical analysis. PS, JB, BE, and ÁV provided us with special expertise in the
15 536 management of celiac disease and inflammatory bowel patients. BC and PK are performing the
16 537 hemorheological measurements and interpreting the results. AH, ÁN, TB, and MTF provided
17 538 us with special expertise in hemostatic and immunological measurements. BK is contributing
18 539 significantly to the biochemical analyses. IV planned and is carrying out the dietary assessment
19 540 of the celiac patients. KM, AS, ZS, and PH are responsible for data management, administrative
20 541 coordination, and biological sampling; they drafted and revised the manuscript. All the authors
21 542 have read and approved the final manuscript.

22 543

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33 554 **COMPETING INTEREST STATEMENT**

34 555 Nothing to declare.

Case Report Form - hemorheological studies

HERMES

Date and time (MM:HH DD:MM:YYYY):

Hematocrit (%):

Red blood cell aggregation (Myrenne aggregometer)¹:

	First sample			Second sample			Mean
M index							
M1 index							

Red blood cell aggregation (LORCA)²:

AI (%): **t ½ (s)**: **γ: (1/s)**

Red blood cell deformability (LORCA)³:

EI ₃₀	EI _{16.87}	EI _{9.49}	EI _{5.33}	EI ₃	EI _{1.69}	EI _{0.95}	EI _{0.53}	EI _{0.3}

Viscosity of whole blood (Brookfield viscometer, mPa·s)⁴:

Viscosity of plasma (Brookfield viscometer, mPa·s)⁴:

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3¹The Myrenne aggregometer (model MA-1, Myrenne GmbH, Roetgen, Germany) consists of a
4 laser diode, a transparent upper plate, and a transparent lower rotating cone (i.e., shearing unit).
5 A sample (30 μl red blood cell suspension) is placed between the gap of the upper plate and the
6 rotating cone. Then, the rotating cone shears the red blood cells at 600 s^{-1} to disaggregate pre-
7 existing aggregates. The cone then suddenly stops moving (**M index or aggregation at stasis**)
8 or continues to rotate (**M1 index or aggregation at low shear**) and shear at 3 s^{-1} while
9 measuring the intensity of transmitted infrared light for 10 s. If the red blood cells tend to
10 aggregate, the light transmittance of the sample increases (as well as M and M1 indices).
11 Measurements are carried out at room temperature three times from two samples.
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14²The Laser-assisted Optical Rotational Cell Analyzer (LORCA, R&R Mechatronics, Hoorn,
15 The Netherlands) consists of a glass cup and a perfectly fitting bob with a 0.3 mm gap in
16 between. The red blood cell suspension is placed in this gap. The disaggregation of the sample
17 is carried out at 500 s^{-1} shear. Following the release, a red laser beam is directed to the sample
18 and the reflection is measured by photodiodes for 120 s. **Aggregation index (AI)** is calculated
19 by the integral of the change in reflection (intensity of light) during the first 10 s corrected to
20 the possible maximal change. $T_{1/2}$ defines the time required for achieving the half of the
21 maximal aggregation. The threshold shear rate is the lowest shear rate that can maintain
22 complete disaggregation (γ). Measurements are carried out at room temperature.
23
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25³Red blood cells are exposed to shear stress on ektacytometry (with LORCA, R&R
26 Mechatronics, Hoorn, The Netherlands), then the change in shape is quantified. LORCA
27 consists of a rotating glass cup with a fitting bob, which provides a nearly homogenous shearing
28 field. Low hematocrit (0.2%) is required for the measurement; therefore, we suspend them in a
29 medium with known viscosity (25 μl blood/5 ml volume). The exposure of red blood cells to
30 shear stress results in the transition of the normally biconcave shape to an ellipsoid one. The
31 deformed cells are examined with a laser beam causing diffraction. The **elongation index (EI)**
32 quantifies the deformation of the red blood cells ($\text{EI}=(A-B)/(A+B)$, where A and B represent
33 the major and the minor axes of the ellipsoids). EI can be determined at various shear stresses,
34 the set of shear stress-elongation index is the so-called ektacytogram. Shear stresses range from
35 0.3 to 30.0 kPa (e.g., **EI_{0.3}**, **EI₃**). Measurements are carried out at room temperature.
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38⁴Viscosity of the whole blood and plasma is determined by Brookfield DV-III Ultra LV
39 Programmable rotational viscometer (Brookfield Engineering Labs; Middleboro, Mass., USA).
40 A spindle is immersed in the sample that the torque required to rotate it is measured. The **torque**
41 is proportional to the viscosity measured at mid-shear (90 s^{-1} or 12 rpm) in our study.
42 Measurements are carried out at 37°C .
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