

DELIVERABLE D.T3.2.5

“Improvement of diagnostics, with testing 3 methods to improve CD practice”





INTRODUCTION

“Improvement of diagnostics with testing 3 methods to improve CD practice”

The idea of PP4 pilot project was to establish whether can noninvasive biomarkers from stool be used for detection and follow-up in celiac disease.

Current pediatric approach is to be as non-invasive as possible. Many invasive procedures are usually done in general anesthesia or deep sedation. Blood sampling is an invasive procedure, and is done only where it cannot be avoided. Diagnostic algorithm for CD involves invasive procedures: blood sampling and often upper endoscopy with multiple biopsies. Follow up is also invasive, periodical blood sampling and specific CD antibodies detection. Since it is a long-life disease, it would be a great improvement to find non invasive procedures.

According to the literature, we wanted to find whether can certain stool biomarkers be used as diagnostic tool in CD diagnosing and follow up. The final idea was to see can fecal elastase and calprotectin be used for CD detection and gluten stool fragments for CD follow up.

We divided patient in 3 groups, the first newly diagnosed CD patients, the second old CD patients with well-controlled CD and the third healthy controls. The goal was to find 50 patients in every group and, since the low incidence of CD in our region and short time period, with the help of partners from Italy who provided stools from newly diagnosed patients. Stools samples were taken from every patient for all 3 biomarkers analysis. Fecal elastase and calprotectin levels were measured in UKC Rijeka laboratory and gluten fragments in laboratory in Trieste. In next pages the procedure and results were explained in details.

Deliverable “Improvement of diagnostics with testing 3 methods to improve CD practice” was prepared on the basis of SUPPORTING TOOLS (templates):

- Report about pilot project ideas & established stakeholder groups
- Pilot Status report
- Pilot final report



DELIVERABLE D.T3.1.1 REPORT ABOUT PILOT PROJECT IDEAS & ESTABLISHED STAKEHOLDER GROUPS

“Improvement of diagnostics, with testing 3 methods to improve CD
practice”



1. Pilot Background

Please describe here the background of your pilot in terms of ideas, preliminary actions, plans defined earlier and methods already chosen, etc. Some of the aspects you can tell about are as follows:

- How did the project idea surface?

Celiac disease is an autoimmune disorder caused by intolerance to gluten-derived peptides of wheat, rice and barley which results in inflammatory injury to the mucosa of the small intestine. The anti-transglutaminase antibodies have greatly simplified diagnosis of celiac disease in daily clinical practice.

Serum concentrations of these antibodies indicate severe enteropathy, suggesting that intestinal biopsy may not be necessary for definitive CD diagnosis. However, paediatricians have to deal with several atypical forms of CD in which the histological assessment of the intestinal biopsy still plays a pivotal diagnostic role.

The aim of our study is to determine the implication of fecal calprotectin, fecal alpha-1-antitrypsin and fecal elastase-1 in the diagnosis, follow-up and response to treatment in celiac disease patients.

- Are there preliminary works that the project is based on? What are they?

- We searched and studied the literature; chose the appropriate biomarkers We selected the study groups, three of them
- We discussed with other partners the study protocol

We were preparing the ethical approval for the study
We contacted the manufacturer of the biomarkers.

- What is the knowledge base behind the project (studies, methods, statistical data etc.)?

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- 4) Alibrahim B, Aljasser MI, Salh B. Fecal calprotectin use in inflammatory bowel disease and beyond: A mini-review. *Can J Gastroenterol Hepatol* 2015;29(3):157-163.



- 5) Poullis A, Foster R, Northfield TC, Mendall MA. Review article: faecal markers in the assessment of activity in inflammatory bowel disease. *Aliment Pharmacol Ther* 2002;16:67581.
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- 7) Capone P, Rispo A, Imperatore N, et al. Fecal calprotectin in coeliac disease. *World J Gastroenterol*.2014;20:611-2.
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- 9) Ertekin V, Selimoğlu MA, Turgut A, Bakan N. Fecal calprotectin concentration in celiac disease. *J Clin Gastroenterol*. 2010;44:544-546.
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- 12) Florent C, L'Hirondel C, Desmazes C, et al: Intestinal Clearance of alpha 1-antitrypsin. A sensitive method for the detection of protein losing enteropathy. *Gastroenterology* 1981;81:777-780
- 13) Crossley JR, Elliott RB: Simple method for diagnosing protein-losing enteropathy. *Br Med J* 1977;1:428-429
- 14) Perrault J, Markowitz H: Protein-losing gastroenteropathy and the intestinal clearance of serum alpha-1-antitrypsin. *Mayo Clin Proc* 1984;59:278-279
- 15) Thomas DW, Sinatra FR, Merritt RJ: Random fecal alpha-1- antitrypsin concentration in children with gastrointestinal disease. *Gastroenterology*. 1981 Apr;80(4):776-82.
- 16) http://www.baspath.co.uk/test_directory/aindex/Alpha1antitrypsin_faecal.htm; accessed on May 8th 2016.
- 17) <http://emedicine.medscape.com/article/931647-overview>; accessed on May 8th 2016.
- 18) Bai JC, Sambuelli A, Niveloni S, Sugai E, Mazure R, Kogan Z, Pedreira S, Boerr L. Alpha 1-antitrypsin clearance as an aid in the management of patients with celiac disease. *Am J Gastroenterol*. 1991 Aug;86(8):986-91.
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- What methods will you / do you plan to use (to motivate stakeholders, to involve lead users, to develop ICT infrastructure, to communicate online etc.)?

Study Type: Observational

Study Design: Case-control

Time Perspective: Cross-sectional



We will have three groups/cohorts of patients:

1. children with newly diagnosed CD
2. healthy children
3. children with previously diagnosed CD on a gluten free diet and negative anti-tTg

Inclusion criteria:

- CD diagnosed in accordance with the criteria of the EPSGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition)
- healthy children without gastrointestinal symptoms and with negative tissue transglutaminase (tTg) antibodies

Exclusion criteria:

- cardiopulmonary, renal, hepatic, neurological, psychiatric, endocrine (including diabetes mellitus and autoimmune thyroiditis) and rheumatologic diseases
- other chronic active intestinal disease
- positive familial history of IBD
- NSAID, proton pump inhibitors and antibiotics consumption
- menstrual or nasal bleeding
- malignancy
- pregnancy
- alcohol misuse

Estimated enrolment: 50 children in each of the three groups

Estimated duration: 9 months

Ages eligible for study: children and adolescents (younger than 18 years)

Genders eligible for study: both

Healthy Volunteers: yes

Sampling method: non-probability sample

2. Pilot Objectives

Please describe here the objectives of your pilot in terms of what the pilot project plans to achieve at the project's end and by what means.



Some of the aspects you can tell about are as follows:

- What are the main outputs of the pilot project (service, process, new management approach, new knowledge...)
- Implication of fecal calprotectin, α 1 antitrypsin and Fecal elastase-1 in the diagnosis, follow-up and response to treatment in celiac disease patients
- Correlation of FC level with the inflammation of the gastrointestinal tract in CD
- Correlation of α 1 antitrypsin and fecal elastase-1 level with the malabsorption in the gastrointestinal tract
- Non-invasive approach at diagnosis and during follow-up
- We want to modernise general paediatric approach to the CD patients
- What is the approach that makes the project viable and sustainable?
 - Through this pilot project, we will try to establish if this non-invasive biomarkers are reliable enough to implement them in everyday practice.
 - These non-invasive methods might be implement in the guidelines for diagnosis and follow-up, if it's utility, cost-effectiveness and impact on the patient's health will be proved.
- What kind of problems are you anticipating and what are your "plan B"-s if something doesn't turn out as you counted in certain situations?
 - If any biomarker in the stool does not show relevant results, in order not to waste money we will stop collecting samples and change to some other relevant biomarker.
 - Non-compliance and drop out of the patients
- Will the pilot have cross-regional impacts? Which are they?
 - We invited other project partners to participate, we got positive responses from: PP2 University medical centre Maribor and PP7 Institute for Maternal and Child Health - IRCCS "Burlo Garofolo"
 - Achievements of our pilot project we will be spread to other project partner and to other healthcare professionals in our country
- Any other aspects you find important ?
 - Non- invasive approach according to now days paediatric practices



3. Partnership

Please describe your stakeholders and their roles in the pilot project. Insert rows according to your needs.

Name	Specialization Area	Role in Project	Motivation / Benefits
<i>If you plan to include a certain type of stakeholder but you don't yet know the specific organization, write "[TBD]" (to be determined) in this column.</i>	<i>Healthcare professional/ patient/presentative of NGO/policy maker...</i>	<i>Participating in development phase/participating in testing, communication, evaluation etc.</i>	<i>What is the main motivation of the organization to participate in the pilot project? What will be their anticipated benefits?</i>
Medical experts (specialists gastroenterologists)	Healthcare professionals	Co-creation and implementation of pilot project Recruitment of the patients and collecting the samples for the analysis	Monitoring and evaluation of newly diagnosed patients and patients on the gluten-free diet Presentation/spreading the data
Laboratory stuff	Healthcare professionals specialist in <i>medical biochemistry</i>	Analysis of the samples	Monitoring and evaluation of newly diagnosed patients and patients on the gluten-free diet
Statistics expert	specialist in medical biochemistry	Statistics analysis of the collected data	Data processing and analysis



4. Business Model Canvas

Please summarize your project plan and approach model described above in this table. Write bullet points in each cell of the table

Key pilot Partners	Key Activities	Value Proposition of the pilot (what is the benefit ?)	End-user (patient) Relationships	End-user (patient) Segments
<p>Project partners</p> <ul style="list-style-type: none"> - PP4 KBC Rijeka - PP7 IRCCS Burlo - PP2 UKC MB <p>Medical experts (specialists gastroenterologists)</p> <p>Immunodiagnostic centre</p>	<ol style="list-style-type: none"> 1. Gathering data 2. Model/method 3 Sampling of Serum and stools 4. Testing of serum 5. testing of stools for 3 biomarkers 6. Clinical and diagnostic data analysis and correlation 7. Evaluation 	<ul style="list-style-type: none"> ▪ Improving the possibilities for coeliac disease diagnosis by non invasive diagnostic biomarkers assays ▪ Improved diagnostic possibilities in serum negative CD antibodies among suspected CD patients. 	<p>Self-service/help/management ?</p> <p>Co-creation process with end-users</p> <p>Partner will be able to provide better service to end users (patients) for identification of disease and timely treatment I selected patients tested negative for classical CD biomarkers.</p>	<p>Young/old/ specific target group</p> <p>Three different study groups will be enrolled:</p> <ol style="list-style-type: none"> 1- children with newly diagnosed CD 2. healthy children 3. children with previously diagnosed CD on a gluten free diet and negative anti-tTg <p>Estimated enrolment: 50 children in each of the three groups</p>
	<p>Key Resources</p> <ol style="list-style-type: none"> 1. Human <ul style="list-style-type: none"> ▪ project partners, health experts 2. Financial <ul style="list-style-type: none"> - PP4 KBC Rijeka - PP7 IRCCS Burlo 		<p>Communication channels ?</p> <ul style="list-style-type: none"> -personal contact - ICT tools -social media -working group 	
Cost Structure		Revenue Streams		



<p>FECAL CALPROTECTIN Fecal calprotectin reagent 30-300 ug/g cca 522 E x4 box. 2.088 E 100-1000 ug/g cca 522 E x4 box. = 2.088 E Preparation kit 152 E x 3 box. = 456 E Kontrol kit 82 E Total Fecal calprotectin cca 4.714 E</p> <p>ALPHA 1 ANTITRIPSIN Reagent 191 E x 4 box = 764 E Standard 194 E Control 201 E React. Puffer 153 E React. Diluent 111 E Total alpha 1 antitripsin 1.423 E</p> <p>FECAL ELASTASE (FE) ELASTASE IN STOOL</p> <p>FE Elisa kit 670 E x 2 box.-1.340 E FE Preparation Kit 51 Ex4box =204 E Total Fecal Elastasa 1.508 E TOTAL 7.645 E</p>	<p>No revenues</p>
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5. Preliminary work plan

Please give a time plan of how you plan to proceed with your pilot project. Define the main stages and milestones of the workflow. Insert rows according to your needs.

Phase Title & Description	Participating Stakeholders	Milestones	Planned Date
<i>Give the title and/or short description of the phase (identification process, focus group meeting, survey, testing... etc.).</i>	<i>According to the Partnership table above. You can write "All" if all of the stakeholders participate in the Phase.</i>	<i>Describe the milestone that you plan to achieve at the end of the phase</i>	<i>Planned date of milestone</i>
1. Searching the literature and preparing the project	Project partners, medical experts	Definition of the aims of the project	6-12/2017, 1/2018
2. Getting the ethical approval	Medical experts	Enable to start with project implementation	2/2018
3. Selection of the patients	Project partners, medical experts	Formation of the three study groups	2-11/2018
4. Informing the patients and signing informed consent	Project partners, medical experts	Enable to start with project implementation Formation of the three study groups	2-11/2018
5. Collecting the data of the included patients	Project partners, medical experts	Preparation for the adequate analysis	10-12/2018
6. Collecting the samples	Project partners, medical experts	Preparation for the adequate analysis	10-12/2018
7. Statistical analysis of the collected data	Healthcare professionals specialist in medical biochemistry	Getting the appropriate and quality data	11-12/2018
8. Comparison of our results with the current knowledge	Project partners, medical experts	Improvement of the quality of the patient's care	12/2018
9. Presentation/spreading the data	Project partners, medical experts	Improvement of the general knowledge	1/2019

ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

Pilot Status Report 1

“Improvement of diagnostics, with testing 3 methods to improve CD practice”





1. Pilot Status According to Objectives defined in D.T3.1.1

- Short description, if pilot development activities are implemented according to objectives set-up in the framework of D.T3.1.1

During the last reporting period we started to collect stool samples. According to the plan, we divided patients into 3 groups with the goal of 50 patients in each group.

The first are newly diagnosed patients who still consume gluten, the second are patients who have CeD and are on gluten free diet, and the third healthy controls.

All team members actively search for eligible patients, along with partners from Italy who collect stool samples from newly diagnosed patients and patients who already have CeD.

In laboratory in UHC Rijeka samples are analyzed for calprotectin and elastase, and in laboratory in Italy for gluten immunogenic peptides. Samples are frozen and transported in portable refrigerator.



2. Activities implemented so far

- Please provide short description of activities implemented so far and explain the progress in developing and testing of pilot solution

From the beginning of the WPT3, we collected approximately half of the needed stool samples. Until the end of September, we collected 40 patients in control group, 17 newly diagnosed patients and 13 patients with known CeD. All of them have calprotectin and elastase levels measured, and the major part have also GIP results finished. Partners from Italy collect the samples from patients with CeD and test all samples for GIP. Patients with new or earlier diagnosed CeD all have anti-tTG and IgA values so the results can be compared with stool samples. After collection of all results, we will be able to make statistical analysis and present final results.

Up till now we haven't found any special problem or deviation.

We already have close coordination with partners from Italy (PP5 and PP7) in collecting stool samples from newly diagnosed and patients with CeD along with laboratory analysis (GIP). If the results of the pilot project show the use of fecal biomarkers, it can be used in every country in hospitals who take care of pediatric CeD patients.

After finishing the pilot project, we will be able to recommend the use of stool biomarkers at the time of diagnosis and during follow up. The algorithm can be made as well as recommendations for the use of additional non-invasive markers. Joint experiences can further improve the use of these markers. The biggest advantage of this approach is its non-invasiveness that can make the compliance of children with CeD and their parents better. It can also improve the quality of life of children with CeD as the result of non-invasiveness.



3. Changes in stakeholder's partnership

There were no changes in stakeholder's partnership.

ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

“Improvement of diagnostics, with testing 3
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Final Report





1. RESULTS ACHIEVED ACCORDINGLY TO OBJECTIVES

- Please review the objectives you have set up in your D.T3.1.1 description, in the Status report Phase 1 and describe activities and results achieved by your pilot. Give an overview of the processes that are part of your pilot project.

The pilot project of PP4 (Deliverable D.T3.2.5) is titled “Improvement of diagnostics, with testing 3 methods to improve CD practice”.

The aim of our first proposal of the pilot project was to determine the implication of fecal calprotectin, fecal alpha-1-antitrypsin and fecal elastase-1 in the diagnosis, follow-up and response to treatment in pediatric celiac disease patients. With the inside of medical literature published after our first pilot project proposal, and because of technical and administrative problems, with consensus of other pilot project partners and with permission of JC, we gave up from our first pilot project proposal. We changed one biomarker: instead of fecal alpha-1-antitrypsin, we measured gluten immunogenic peptides in stool samples.

Our study was conducted to compare fecal calprotectin, fecal elastase-1 and gluten immunogenic peptides levels in 22 untreated (i.e. newly diagnosed) CD patients with 45 children with previously diagnosed celiac disease on a gluten free diet and with a control group of 57 healthy children.

As fecal calprotectin is a granulocyte cytosolic protein considered to be a promising marker of subclinical inflammation of the gastrointestinal tract, the idea was to correlate fecal calprotectin levels with the inflammation of the gastrointestinal tract in CD in three defined groups.

The study found no statistically significant difference between FC levels in newly diagnosed CD patients, those on gluten free diet and controls.

We have shown no comparison between FC levels of 22 untreated paediatric CD patients and control group of 57 healthy subject.

According to our study, there is no evidence to support the use of FC as a biomarker of subclinical inflammation in CD, i.e. the use of FC as a diagnostic biomarker for CD in the paediatric population. Randomly measured FC levels in CD patients are within normal levels, and elevation of FC should not be explained by CD and clinician should do the further investigation for inflammatory bowel disease.

Considering protein loss in celiac disease, as a result of breakdown of enterocyte barrier with villous atrophy, we were assuming that the measuring of fecal alpha-1-antitrypsin could be used for the estimation of enteric protein loss in CD patients.

In 24% (11/46) patients with previously diagnosed CD, we found decreased levels of fecal elastase-1. In healthy children we also found decreased levels of fecal elastase-1 in 21% (12/56) subjects. According to these small sample size data, fecal elastase-1 is unspecific biomarker and it is not helpful in diagnosis, follow-up and response to treatment in paediatric celiac disease patients.

Detection of gluten immunogenic peptides in faeces have been proposed as new non-invasive biomarkers to detect gluten intake and to verify gluten free diet compliance in CD patients. Gluten immunogenic peptides identify voluntary or unknown consumption of gluten within 48 to 72 hours after the transgression.

The gluten immunogenic peptides test in faeces (by ELISA) was positive in 20/22 samples of the newly diagnosed patients and in 25% (14/56) previously diagnosed CD patients who should follow gluten free diet. These results showed that every 4th CD patient is not strictly adherent to the gluten free diet. Two negative results in first group of patients should raise awareness, to the patients and to the physicians, that patients must me on gluten containing diet in the time serology tests are done, to avoid the false negative results.



2. ADDED VALUE OF THE DEVELOPED & TESTED PILOT SOLUTION IN YOUR REGIONAL ENVIRONMENT

- Please describe shortly, what is the gained added value for the end-user of pilot service solution

ADDED VALUE for END-USER

Short term effects and long-term effects	
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1. To determine the implication of fecal calprotectin, fecal elastase-1 and gluten immunogenic peptides in the diagnosis, follow-up and response to treatment in celiac disease patients
 2. To establish if this non-invasive biomarkers are reliable enough to implement them in everyday practice
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3. DEVIATION AND PROBLEMS ENCONTERED

- In case your outcomes are different from the planned, please give an explanation of the reasons and formulate your modified results achieved. Was your planned model working or did you had to make modifications, if yes, describe? Did you had any problems in you pilot implementation? If yes, which was the solution adopted?

- With the inside into the medical literature published after pilot project proposal in AF, and because of the constructive critics of other project partners we gave up from our initial pilot project proposal. Instead of measurement of of fecal calprotectin, fecal alpha-1- antitrypsin and fecal elastase-1 in stool sampels of newly diagnosed CD patients, patients with already known CD and control group, we measured calprotectin, fecal elastase-1 and gluten immunogenic peptides (GIP) in stool samples.

- Since we had no possibility to test stool samples for GIP, partners from Italy tested all stool samples for GIP.

- We had no enough newly diagnosed CD patients in examined period, so we analyzed stool samples of newly diagnosed patients from Italy.

4. LESSON LEARNED RELATED TO CO-CREATION OF PILOT SOLUTIONS WITH ENGAGED STAKEHOLDERS

- Please describe what were the benefits and setbacks related to co-creation of pilot project with stakeholders.

LESSONS LEARNED

Benefits

1. easiness of collecting materials

2. parents' wiliness for giving the samples

3.

5. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

- What are your further activities of the pilot project development?

We wanted to see whether can non invasive way be used in children with newly diagnosed CD and children already having CD. We tried to find can certain biomarkers in stool be used instead of invasive blood sampling. The idea was to try to give our results as proposal for CD diagnosis and follow up non only to local level, but also to national and even international level. Results found in literature were promising enough to start our pilot project. Unfortunately, the results were negative in two out of three conditions and didn't prove their use in everyday practice. The reason for this was maybe in shortness of time for collecting bigger groups of patients; maybe on bigger sample the results could be different. Anyway, we cannot give any recommendation for the continuation of the fecal elastase 1 and calprotectin biomarkers' usage in everyday clinical practice. As the results weren't positive, we won't continue with using neither of the two biomarkers.

The only one biomarker that showed usefulness is gluten immunogenic peptides for detecting low adherence to gluten free diet. It can be useful in children ad non-invasive follow up as it is simple to collect and very well follows gluten ingestion. There are more studies needed to see what is the time distance of detecting GIP in stool and serological biomarkers to start to raise and to establish the right time interval for stool analysis. We are interested to see whether can our laboratory start to detect GIP in stool as we are willing to continue with the GIP detection in bigger sample and longer time period.