Interreg CENTRAL EUROPE
Focus IN CD
European Union
European Regional Development Fund

DELIVERABLE D.T3.2.4

IMPROVEMENT OF EARLY DIAGNOSTICS, TESTING METHOD ´IGA T-TG DEPOSITS IN TISSUE SAMPLE´

1. INTRODUCTION

Coeliac disease can be diagnosed with combination of different tests. In majority of cases, patients must undergo upper endoscopy – Esophagogastroduodenoscopy (EGDS), whereby biopsy specimens from multiple parts of distal duodenum and duodenal bulb are obtained. Pathologists further examine these samples and determine the degree of intestinal damage typical of coeliac disease. In majority of cases, the diagnosis can be firmly established with currently available diagnostic tests. However, in some cases the tests can yield unclear or conflicting results. In such cases, clinicians currently cannot reliably diagnose the disease and patients are thus not treated properly. In order to diagnose the disease in these patients, new techniques should be used. Determination of early immunological reactions in the intestinal tissue can enable pathologists to determine whether celiac disease specific reaction against gluten is present or not. One such test is the determination of the presence of specific intraepithelial lymphocytes that are specific of coeliac disease (gamma/delta IELs). Another possibility is the determination of coeliac disease specific antibodies in the intestinal tissue itself. Determining tissue deposits of t-TG antibodies was introduced recently and is only used in very few centres worldwide. One of the project partners IRCCS Burlo Garofolo has experience with this method. The aim of this activity was to **apply a new diagnostic method** at University Medical Centre Maribor, which will **improve service in coeliac disease diagnosis**.

2. PILOT PROJECT IDEAS & ESTABLISHED STAKEHOLDER GROUPS

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?*
  - Coeliac disease can be diagnosed with combination of different tests. In majority of cases, the diagnosis can be firmly established with currently available diagnostic tests. However, in some cases the tests can yield unclear or conflicting results. In such cases, clinicians currently cannot diagnose the disease and a patient thus is not treated properly.
  - In order to diagnose the disease in these patients some new techniques could be used. Determination of early immunological reactions in the intestinal tissue can enable pathologists to determine whether celiac disease specific reaction against gluten is present or not.
  - One such test is the determination of the presence of specific intraepithelial lymphocytes that are specific of coeliac disease (gamma/delta IELs).
  - Another possibility is the determination of coeliac disease specific antibodies in the intestinal tissue itself. Determining tissue deposits of t-TG antibodies was introduced recently and is only used in very few centres worldwide.

- *Are there preliminary works that the project is based on? What are they?*
  - Experience of one of the partners with IgA t-TG intestinal deposits.

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

  Several studies have addressed the advantages of detection of tissue deposits of t-TG in coeliac disease.
  - Borrelli M, Maglio M, Korponay-Szabó IR, et al. Intestinal anti-transglutaminase 2 immunoglobulin A deposits in children at risk for coeliac disease (CD): data from the PreventCD study. Clin Exp Immunol. 2017
  - Maglio M, Ziberna F, Aitoro R, et al. Intestinal Production of Anti-Tissue Transglutaminase 2 Antibodies in Patients with Diagnosis Other Than Celiac Disease. Nutrients. 2017
  - Gatti S, Rossi M, Alfonsi S, Mandolesi A, Cobellis G, Catassi C. Beyond the Intestinal Celiac Mucosa: Diagnostic Role of Anti-TG2 Deposits, a Systematic Review. Front Med (Lausanne). 2014
  - Tosco A, Auricchio R, Aitoro R, et al. Intestinal titres of anti-tissue transglutaminase 2 antibodies correlate positively with mucosal damage degree and inversely with gluten-free diet duration in coeliac disease. Clin Exp Immunol. 2014

- Koskinen O, Collin P, Korponay-Szabo I, Salmi T, et al. Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. J Pediatr Gastroenterol Nutr. 2008

- *What methods will you / do you plan to use (to motivate stakeholders, to involve lead users, to develop ICT infrastructure, to communicate online etc.)?*
  - We plant to use existing infrastructure in UKC-MB to collect tissue samples. For this purpose, we will use existing endoscopes.
  - We plan to purchase liquid nitrogen containers in which we will store the samples for further analysis.
  - We plan to purchase small portable liquid nitrogen container for transport of samples within our institution from endoscopy room to storage facility.
  - We plan to purchase portable freezer for transport of samples to a distant location.
  - Samples will be transported with portable freezer to partner IRCCS Burlo location in Trieste.
  - One of the members of UKC MB project team will learn the technique of t-TG deposits determination in tissues with the help of partner IRCCS Burlo.
  - We will transfer the expertise to our institution within which we will use existing infrastructure: cryotome for cutting the samples, immunology laboratory where we will stain the samples, and use immunofluorescence method for detecting the positive or negative samples.
  - We will compare our results with other diagnostic methods in patients.

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…)?*
  - Applying a new diagnostic method at one of the institutions.
  - Improved service in coeliac disease diagnosis.

- *What is the approach that makes the project viable and sustainable?*
  - Building on an existing infrastructure will decrease the cost of introduction of the new method.
  - Role of the partner as a reference centre for coeliac disease will enable partner to provide service for other institutions in the region.
  - Equipment purchased within the project will remain to be used after the project.

- What kind of problems are you anticipating and what is your "plan B"-s if something does not turn out as you counted in certain situations?
  - Inability to collect enough samples is possible, however increased number of patients make this risk very small.
  - Inability to learn the new technique is possible but very unlikely issue.
  - Lack of skilled personnel is possible issue; however, UKC has many experts that can take over this task.

- Will the pilot have cross-regional impacts? Which are they?
  - We will provide the improved service to other hospitals within the region and to other health care institutions. We will involve other clinical partners from other hospitals.
  - We will present our experience at international meetings to other potential stakeholders.

- Any other aspects you find important.
  - Implementing new methods in institution is always a driving force for new developments in other fields.

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
If you plan to include a certain type of stakeholder but you do not yet know the specific organization, write "[TBD]" (to be determined) in this column.	*Healthcare professional/ patient/presentative of NGO/policy maker…*	*Participating in development phase/participating in testing, communication, evaluation etc.*	*What is the main motivation of the organization to participate in the pilot project? What will be their anticipated benefits?*
1. Medical experts - specialists gastroenterologists, pathologists	Healthcare professionals	Co-creation, design and implementation of pilot project's main activities	Improved diagnostic possibilities, improved knowledge, better service
2. Medical experts – clinical biochemistry and immunology	Healthcare professionals	Co-operation with development of appropriate laboratory techniques	Improved diagnostic possibilities, better service
3. Coeliac disease society	NGOs	End beneficiaries	Better service

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

- Project partners (UKC-MB, IRCCS Burlo)
- Slovenian Celiac society (SDC)
- Medical experts (specialists gastroenterologists, immunologist, biochemists)

Key Activities

1. Sampling of tissue.
2. Freezing of samples
3. Sample storing
4. Learning new technique at partner IRCCS
5. Transferring of the technique to UKC MB
6. Testing of new technique

Key Resources

1. Human
   - project partners, health experts
2. Financial
   - UKC-MB

Value Proposition of the pilot (what is the benefit?)

- Implemented one new diagnostic method
- Improving the possibilities for coeliac disease diagnosis
- Improved possibilities to detect disease at early stage
- Improved diagnostic possibilities in unclear cases.

End-user Relationships

• Partner will be able to provide better service to end users (patients)

Communication channels?

- personal contacts
- web based communication
- fieldtrip

End-user Segments

At least 15 patients diagnosed with the help of new method

Cost Structure

Pilot development coordination costs: distribution of working hours

Maintenance costs

Revenue Streams

Not planned

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
Give the title and/or short description of the phase (identification process, focus group meeting, survey, testing… etc.).	*According to the Partnership table above. You can write "All" if all of the stakeholders participate in the Phase.*	*Describe the milestone that you plan to achieve at the end of the phase*	*Planned date of milestone*
1.Purchase of liquid nitrogen containers	Project partner UKC-MB	Containers purchased	August 2017
2.Purchase of transport freezer	Project partner UKC-MB	Freezer purchased	August 2017
3. Storage of samples	Project partner UKC-MB	30 samples frozen	September 2018
4. Learning the new technique at IRCCS Burlo	Project partner UKC-MB, IRCCS Burlo	New technique learned	September 2018
5. Transferring of new method to UKC-MB	Project partner UKC-MB, IRCCS Burlo	New technique implemented in UKC-MB	January 2019
6. Testing of real patient samples at UKC	Project partner UKC-MB	Samples tested	February-March 2019
3. Analysis of results	Project partners UKC-MB, IRCCS Burlo, Coeliac disease society	Analysis of results	March-April 2019

3. IMPLEMENTATION OF PILOT PROJECT - Pilot Status Report 1

Testing of real patient samples was done during this period with the close collaboration of partners PP2, PP4 and PP7. Samples are still collected and stored at endoscopy unit of PP2. One of the members of PP2 team (Petra Rižnik) spent one month at the IRRCS Burlo Garfolo laboratory, where she tested patient samples with the new method and learned all the details of the technique, which will be later transferred to PP2 and with that to Slovenia. PP4 and PP7 provided the information about the needed reagents, provide the protocol for tissue samples testing, and helped in providing information needed to establish new method in a new institution.

Samples tested during this period proved the usefulness of the method. In one of the patients who had equivocal results of standard celiac disease specific tests, the method proved the presence of deposits of celiac disease specific antibodies in the intestinal tissue, which proved the existence of the disease.

Plans

Purchase of all necessary reagents and materials to set-up the technique at Immunology laboratory at PP2. Co-operation of Immunology and pathology laboratory. Further collection of samples. Final testing of samples.

4. 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.**

Coeliac disease can be diagnosed with combination of different tests. In majority of cases, patients must undergo upper endoscopy – Esophagogastroduodenoscopy (EGDS), whereby biopsy specimens from multiple parts of distal duodenum and duodenal bulb are obtained. Pathologists further examine these samples and determine the degree of intestinal damage typical of coeliac disease. Coeliac disease can be diagnosed with combination of different tests. In majority of cases, the diagnosis can be firmly established with currently available diagnostic tests. However, in some cases the tests can yield unclear or conflicting results. In such cases, clinicians currently cannot reliably diagnose the disease and patients are thus not treated properly. In order to diagnose the disease in these patients new techniques could be used. Determination of early immunological reactions in the intestinal tissue can enable pathologists to determine whether celiac disease specific reaction against gluten is present or not. One such test is the determination of the presence of specific intraepithelial lymphocytes that are specific of coeliac disease (gamma/delta IELs). Another possibility is the determination of coeliac disease specific antibodies in the intestinal tissue itself. Determining tissue deposits of t-TG antibodies was introduced recently and is only used in very few centres worldwide. One of the project partners IRCCS Burlo Garofolo has experience with this method. The aim of this activity was to **apply a new diagnostic method** at University Medical Centre Maribor, which will **improve service in coeliac disease diagnosis**.

In order to introduce this new method we used existing infrastructure in UKC-MB to collect tissue samples with existing endoscopes. We purchased liquid nitrogen containers in which we stored samples form CD patients for further analysis. We purchased small portable liquid nitrogen container for transport of samples within our institution from endoscopy room to storage facility, and a portable freezer for transport of samples to a distant location. This freezer was used to transport samples to collaborate partner IRCCS Burlo Garofolo in Trieste. One of the members of UKC MB project team Dr. Petra Rižnik learnt the technique of t-TG deposits determination in tissues with the help of partner IRCCS Burlo Dr. Tarcisio Not and Dr. Luigina DeLeo. The expertise was then transferred to our institution within which we used existing infrastructure: cryotome for cutting the samples, immunology laboratory to stain the samples, and immunofluorescence method for detecting the positive or negative samples. The results of the staining were compared with other diagnostic methods in patients. Results showed a great concordance with standard diagnostic methods, and proved to be very helpful in selected patients with discrepant standard tests.

5. 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	Long-term effects
1. Introduction of new diagnostic method.	1. Improved diagnostic capacities for celiac disease of University Medical Centre Maribor and Slovenia.
2. Clarification of diagnosis in selected complicated cases.	2. Improved health outcome for patients with difficult diagnosis.
	3. Improved service of health care sector for patients with celiac disease.

6. DEVIATION AND PROBLEMS ENCOUNTERED

- **In case your outcomes are different from the planned, please explain 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?**

We have encountered few problems during the pilot activity, and adopted necessary mitigation measures.

- Number of samples varied during the duration of the study, however with other activities of the project level of new patients was high enough.
- Lack of skilled personnel within the institution proved to be a minor problem since UKC MB has enough experts who can take over this task.
- Specific steps have to be performed at different locations within the UKC MB, therefore a good coordination of all stakeholders is crucial.
- Obtaining appropriate laboratory material for new technique is an important point, and it is overcome with the help of centre with long experience (IRCCS Burlo Garofolo).

7. 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	Setbacks
1. Involvement of multidisciplinary team of experts from different units in the UKC MB allowed exchange of expertise.	1. Involvement of larger groups of experts can slow a learning curve. Members of the team with less motivation can jeopardise introduction of novel methods.
2. Cooperation of two partners with different backgrounds and availability of equipment and material enabled faster adoption of new technique.	2.
3. Feedback from other members of multidisciplinary team helped in optimising the method in local settings.	3.

8. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

- What are your further activities of the pilot project development,
  - On the local level?
  - Method will be used at the University Medical Centre Maribor in future especially in difficult cases of celiac disease. Methodology will be updated regularly based on new knowledge and reports. New members will be invited to learn the technique. Method will be available for other centres in the region and in the country.
  - On transnational level?
  - Method will be used at the University Medical Centre Maribor in future. It will be updated with the help of the partners form IRCCS Burlo Garofolo, and the data of difficult patients will be exchanged between centres. Results will be presented at international meetings and new techniques will be made available to other centres in neighbouring countries. Trainees form other countries will be presented the technique.

- How did you plan to ensure sustainability to your pilot? Have you plan any action for the maintenance/follow up/development of the actions implemented, after the project ends?

Building on an existing infrastructure will decrease the cost of introduction of the new method in the institution and will enable its long-term use. Since UKC Mb serves as a reference centre for coeliac disease this service will provided to other institutions in the region. All the equipment purchased within the project will remain to be used after the project to store samples and to perform analysis.