

DELIVERABLE D.T3.2.8

“ Improvement of diagnostic of atypical CD patients ”
PP7 IRCCS Burlo Garofolo





INTRODUCTION

“Improvement of diagnostic of atypical CD patients”

Pilot activity is being developed by the PP7 partner IRCCS Burlo Garofolo Trieste. We investigated whether histological analysis of the duodenal biopsies combined with intestinal IgA anti-transglutaminase deposits immunoassay makes CD diagnosis possible in at-risk children with low concentrations of serum anti-transglutaminase antibodies and normal intestinal mucosa.

Our activities performed during the pilot are listed below:

Two hundred forty-five symptomatic children positive for serum anti-tTG (>7 U/mL) were enrolled and divided into 3 groups: extensive duodenal atrophy (n=209), with IgA anti-tTG deposits throughout the duodenum and high serum anti-tTG concentrations (157±178 U/mL); bulb duodenal atrophy (n=22), with widespread IgA anti-tTG deposits in 9 and in the bulb alone in 13 and low serum anti-tTG concentrations (13.9±8.7 U/mL); and normal duodenum (n=14), with widespread IgA anti-tTG deposits in 8 and in the bulb alone in 6 and low serum anti-tTG concentrations (10.6±6.2 U/mL). All patients in the first 2 groups were diagnosed with CD and 8 from the third group. All improved after 1 year of gluten-free diet. Bulb duodenal analysis led to a 12% (30/245) increase in CD diagnosis. No CD-related lesions were observed in the 30 control subjects.

Conclusions: In children at risk for CD, bulb duodenum biopsy sampling is essential to identify villous atrophy and detect IgA anti-tTG deposits even in absence of intestinal lesions. These mucosal autoantibodies could well represent a new standard for diagnosing CD.

We have shared these results with the gastroenterologists of our region in two meetings and promoted a stable collaboration for the diagnosis of the patients described above and we proposed that this diagnostic-procedure should be included in the clinical practice . We have also published these original data (Gastrointest Endosc 2018;88:521)

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

Pilot project Start-up description
template

Version 1
02 2017

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?

- In the last few years clinical picture of celiac disease (CD) has radically changed with a wide spectrum of clinical presentation ranging from classical gastro-intestinal or extra-intestinal complaints to asymptomatic forms. Along with these clinical changes a broad spectrum of intestinal damage from clear intestinal atrophy to normal intestinal mucosa are observed. Furthermore, some symptomatic subjects were tested negative for serum anti-transglutaminase antibodies (anti-tTG), making it difficult to diagnose CD.
- These observations strongly support the idea that a **subclinical form of genetic gluten intolerance may exist** with apparently normal intestinal mucosa and with normal serum anti-TG concentrations.
- In the context of very high clinical and biological variability of CD, the **constant and specific CD marker seems to be the mucosal intestinal deposits of IgA anti-tTG** which disappear during the gluten-free diet.

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

- Our group's experience in the diagnosis of CD in subjects lacking celiac markers: normal biopsy and absence of serum anti-tTG antibodies.

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

- Several reports have addressed the usefulness of intestinal anti-tTG detection for CD diagnosis in patients lacking celiac markers.
- Kaukinen K, Peraaho M, Collin P, et al. Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study. *Scand J Gastroenterol.* 2005;40:564-572.
- Not T, Zibera F, Vatta S, et al. Cryptic genetic gluten intolerance revealed by intestinal antitransglutaminase antibodies and response to gluten-free diet. *Gut.* 2011;60(11):1487-1493.

- Auricchio R, Tosco A, Piccolo E, et al. Potential Celiac Children: 9-Year Follow-Up on a Gluten-Containing Diet. *Am J Gastroenterol.* 2014;109(6):913-921.
 - Borelli M, MaglioM, Korponay-Szabo I et al Intestinal antitransglutaminase 2 immunoglobulin A deposits in children at risk for CD: data from the prevent CD Study. *Clin Exp Immunol* doi:10.1111/CEI.13078
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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.)?**
- We plan to use existing infrastructure and expertise in Burlo Garofolo children hospital to collect and to analyze biopsy samples for the intestinal anti-transglutaminase antibodies in selected patients suffering from CD symptoms but negative for CD related biological markers.
 - We will transfer our clinical and diagnostic knowledge on the diagnosis of celiac disease to other medical centers in our region or in other Italian regions.
 - We have planned the spread of our celiac disease immunodiagnostic technologies to the UKC-MB partner.
 - We will compare the standard diagnostic procedures with our immune-assays for the CD diagnosis

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...)**
- - Applying a new diagnostic methods among regional or extraregional gastroenterological units
 - To consolidate clinical diagnostic collaborations with the partners of the present project
- **What is the approach that makes the project viable and sustainable?**
 - Role of the partner as a reference centre for the diagnosis of celiac disease in all its clinical and biological forms.
 - The presence of an equipment able to carry out the required analyzes
- **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?**
 - The impossibility to involve regional or extra-regional centers in the advanced diagnosis of celiac disease is possible but very a unlikely issue.
- **Will the pilot have cross-regional impacts? Which are they?**
 - We will consolidate the diagnostic improvement of celiac disease for other hospitals in our region and also for medical centers outside our region

- We will present our experience at international meetings and to other potential stakeholders
- **Any other aspects you find important ?**
- To build and strengthen a network between the hospitals of our region on this specific issue of and also on other topics of gastroenterology.

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</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</i>

<i>organization, write "[TBD]" (to be determined) in this column.</i>			<i>their anticipated benefits?</i>
1. 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
2. Laboratory stuff	Healthcare professionals specialist in medical biochemistry	Analysis of the samples	Monitoring and evaluation of newly diagnosed patients and patients on the gluten-free diet
3. 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
<ul style="list-style-type: none"> • Project partners: PP7 IRCCS Burlo; PP5 UNITS; PP2 UKC MB) • Medical experts (specialists gastroenterologists, allergologist, dietician) • Immunodiagnostic center (FVG region hospitals) 	<ol style="list-style-type: none"> 1. Gathering data 2. Model/method 3 Sampling of Serum and tissue 4. Testing of serum 5. testing tissue samples 6. Clinical and diagnostic data analysis and correlation 7. Evaluation 	<ul style="list-style-type: none"> • Improving the possibilities for coeliac disease diagnosis • Improved possibilities to detect disease at early stage • Improved diagnostic possibilities in atypical or asymptomatic cases. 	<ul style="list-style-type: none"> • Partner will be able to provide better service to end users (patients) for earlier identification of disease and timely treatment. 	<p>Approximately 400 suspected CD patients tested.</p> <p>Approximately 40 clinically atypical CD patients identified.</p>

<p>Key Resources</p> <p>Key Resources</p> <p>1. Human</p> <ul style="list-style-type: none"> • project partners, health experts <p>2. Financial</p> <ul style="list-style-type: none"> • PP7 BURLO • PP5 UNITS 		<p>Communication channels ?</p> <ul style="list-style-type: none"> -personal contact - ICT tools -social media -working group
<p>Cost Structure</p> <p>Pilot development coordination costs: distribution of working hours</p> <p>Maintenance costs</p>	<p>Revenue Streams</p> <p>Not planned</p>	

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	Project partners, medical experts	Improvement of the quality of the	12/2018

current knowledge		patient's care	
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

“ Improvement of diagnostic of atypical CD patients ” PP7 IRCCS
Burlo Garofolo-Trieste

Status Report 1



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**

Pilot development activities are implemented according to objectives set-up in the framework.

The aim of this study is to investigate whether histological analysis of the duodenal biopsies combined with intestinal IgA anti-transglutaminase deposits immunoassay makes CD diagnosis possible in at-risk children with low concentrations of serum anti-transglutaminase antibodies and normal intestinal mucosa.

- **The approach that makes the project viable and sustainable:**
 - We have preliminary data confirming that intestinal IgA anti-transglutaminase deposits immunoassay makes CD diagnosis possible in symptomatic children tested negative for serum CD-auto-antibodies.
 - By putting together different expertise such as family paediatrician, gastrointestinal-paediatrician and biotechnologist will speed up patients' collection and analysis
 - As a reference centre for coeliac disease will enable partner to provide service for other institutions in the region.

- **We anticipated the following problems:**

- None at the moment

- **Did the development process contribute to any additional new objectives?**

No, we anticipated all objectives.

- **Did the team discover that any of initially set-up objective would not be reachable and please explain reasons/circumstances?**

NO problem foreseen



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

We have defined the project working group composed by:

- PP5 laboratory of applied Biology
- Family pediatricians, pediatric gastroenterologists of the North-East of Italy (FVG, Veneto, Lombardia)

We have organized several meetings:

- on May 2018 in Trieste “**FOCUS IN CD dissemination**”

The meeting was organized as a training events for young pediatricians, nutritionists, food technologists and researcher.

- PP7 organized together with the PP5 partner **two important meetings with over 500 pediatricians and family doctors from our region (Grado 11 October 2018) and Veneto (Treviso 16 November 2018)** to improve the knowledge regarding the diagnosis and follow-up of celiac disease. In particular, in these meetings an operative protocol was proposed and shared to include the diagnosis of CD by new immune-assay based on the detection of CD-auto-antibodies on the intestinal biopsies.

From the practical point of view The PP7 Two hundred forty-five symptomatic children positive for serum anti-tTG (>7 U/mL) were enrolled and divided into 3 groups: extensive duodenal atrophy (n=209), with IgA anti-tTG deposits throughout the duodenum and high serum anti-tTG concentrations (157 ± 178 U/mL); bulb duodenal atrophy (n=22), with widespread IgA anti-tTG deposits in 9 and in the bulb alone in 13 and low serum anti-tTG concentrations (13.9 ± 8.7 U/mL); and normal duodenum (n=14), with widespread IgA anti-tTG deposits in 8 and in the bulb alone in 6 and low serum anti-tTG concentrations (10.6 ± 6.2 U/mL). All patients in the first 2 groups were diagnosed with CD and 8 from the third group.

With these results we discussed with pediatric-gastroenterologists and family pediatricians of our region and of Veneto region to adopt a common guideline to diagnose CD among patients with atypical CD presentation .

3. Changes in stakeholder’s partnership

NO changes in the STAKEHOLDERS’s partnership was monitored during the initial phase of the project

ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

D.T3.2.8 Improvement of diagnostic of atypical CD
patients
Pilot project Final Report PP7 Burlo Garofolo





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.

Our Pilot project “Improvement of diagnostic of atypical CD patients” comprises 3 topics:

1) Application and verification of new immunological technique in the diagnosis of celiac disease (CD) in all its clinical presentation forms in our clinical practice.

- Activity: we investigated whether histological analysis of the duodenal biopsies combined with intestinal IgA anti-transglutaminase deposits immunoassay makes CD diagnosis possible in at-risk children with low concentrations of serum anti-transglutaminase antibodies and normal intestinal mucosa.

- Results: Two hundred forty-five symptomatic children positive for serum anti-tTG (>7 U/mL) were enrolled and divided into 3 groups: extensive duodenal atrophy (n=209), with IgA anti-tTG deposits throughout the duodenum and high serum anti-tTG concentrations (157±178 U/mL); bulb duodenal atrophy (n=22), with widespread IgA anti-tTG deposits in 9 and in the bulb alone in 13 and low serum anti-tTG concentrations (13.9±8.7 U/mL); and normal duodenum (n=14), with widespread IgA anti-tTG deposits in 8 and in the bulb alone in 6 and low serum anti-tTG concentrations (10.6±6.2 U/mL). All patients in the first 2 groups were diagnosed with CD and 8 from the third group. All improved after 1 year of gluten-free diet. Bulb duodenal analysis led to a 12% (30/245) increase in CD diagnosis. No CD-related lesions were observed in the 30 control subjects.

- Conclusions: In children at risk for CD, bulb duodenum biopsy sampling is essential to identify villous atrophy and detect IgA anti-tTG deposits even in absence of intestinal lesions. These mucosal autoantibodies could well represent a new standard for diagnosing CD.

We have shared these results with the gastroenterologists of our region in two meetings and promoted a stable collaboration for the diagnosis of the patients described above. We have also published these original data (Gastrointest Endosc 2018;88:521) and declared the support of the Interreg Central Europe “Focus in CD” project.

2) Transferability of these innovative immunological techniques to other partners of the Focus in CD project.

- Activity: in order to spread this diagnostic technique among the partners of the present project we have made available our activity to analyze intestinal biopsy specimens and teach this immunological technique to people belonging to the other partners.

- Results: We received 100 intestinal biopsies from the pediatric gastroenterology of Maribor University Medical Center (PP2) and assessed the level of sensitivity and diagnostic specificity of the test which was 100%. At our laboratory, Petra Rižnik MD. (PP2’s resident of pediatrics) spent a month where she followed a training to look for anti-ttg antibodies on 10 intestinal biopsies of subjects with celiac disease, learning all the operative steps of the immunological test. In this way this technique has been successfully transferred to the PP2’s pediatric gastroenterology.



3) Sharing of these diagnostic techniques among pediatric gastroenterology centers in northeastern Italy.

- Activities: involve pediatricians and adult gastroenterologists in the existence of different clinical and biological forms of CD and the possibility of recognizing all these forms with immunological techniques now available. Promote the formation of stakeholders groups in the care of these CD subjects.

- Results: we have organized several scientific meetings where we have illustrated the new clinical forms of CD and the new immunological techniques able to identify these conditions. In addition, with the support of the Italian Celiac Association (AIC), we have established a stable working group of pediatricians and adult gastroenterologists from our region and of the north-east Italy (gastroenterological units of Trieste, Udine, Aviano, Pordenone, Gorizia, Treviso, Padova, Vicenza, Monza) to share all the "difficult" cases of CD. Recently, a study project entitled "Use of intestinal anti-transglutaminase antibodies in the diagnosis of celiac disease in pediatric age and in the adult: multicentric prospective study" was carried out and funded by our Institute to give continuity to this collaboration.

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	Long-term effects
1. We have identified some clinical forms of celiac disease and above all verified a specific CD diagnostic pathway. We have shared this knowledge with pediatricians and gastroenterologists now able to recognize and manage these new CD-clinical conditions.	1. Repeat of the meetings in our institution and alerting HCPs and stakeholders to share CD-diagnostic guidelines will strengthen the level of CD-medical care.
2. The interaction with other partners has led to the dissemination of immunological techniques for the best medical practice in the CD-diagnosis.	2. This collaboration, inaugurated by the present project, will allow an effective collaboration on CD and the drafting of other bilateral projects.
3. Increased knowledge on the presence of different CD-clinical forms, their recognition with an appropriate diagnostic path, and the medical care in daily clinical practice.	3. The stable presence of stakeholder groups on this specific field will allow the continuity of the knowledge acquired during this project.



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?

We have no particular problems in the implementation of our pilot project. This is due to the high interest of pediatricians and gastroenterologists involved in the project but also from the association of patients and their families that keep alive the interest on this disease both to ensure an early diagnosis and a good medical care.



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	Setbacks
1. Collaboration with pediatricians and adult gastroenterologists has been extremely productive and useful in daily clinical practice and has generated a stable collaboration never before experienced.	1. There were no setbacks in bringing our project to a successful conclusion.
2.	2.
3.	3.

5. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

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

On the local level ? We intend to maintain a constant interaction with the groups of pediatricians and adult gastroenterologists who have been trained in this project with two annual meetings with the discussion of clinical cases and sharing innovative CD-diagnostic methods.

On transnational level ? we intend to extend this pilot project model to other working groups on celiac disease, which are present in European scientific societies.

- 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?
- Working groups: the presence of working groups (pediatricians and adult gastroenterologists) born during the project will ensure the continuity of the new clinical and diagnostic features acquired. The acquisition of diagnostic techniques and the knowledge of the various clinical forms of celiac disease will allow some groups of this project to extend this knowledge to other Italian regions and work groups.
- Guidelines: the acquired clinical and diagnostic experience will allow to formulate and apply innovative guidelines for CD treatment by the various working groups.