

DELIVERABLE D.T3.2.6

“Detecting and managing CD patient within a 'cohort of super allergic population”





INTRODUCTION

“Detecting and managing CD patient within a 'cohort of super allergic population

Pilot activity is being developed by the PP5 partner University of Trieste, in cooperation with PP7 partner Burlo Garofolo Trieste

We have preliminary data confirming that super allergic patients have an increase frequency of CD, by putting together different expertise such as allergologists, GI and biotechnologist our aim was to speed up patients' collection and analysis to confirm data.

The pilot had the aim to identify a new clinical condition at risk of CD, which calls for screening in other hospitals within the region and to other health care institutions in order to prevent possible complications of untreated CD.

Our activities performed during the pilot are listed below:

- we have enrolled a total of one hundred and twenty children (72 M, 48 F, mean age 10 ± 3 years) with very severe food allergy (98 with cow's milk allergy, 48 with egg allergy, 15 with wheat allergy).

Serum samples were analyzed

- for IgA anti-endomysium antibodies (AEA)
- IgA-IgG anti-TG2 antibodies
- The susceptibility alleles for CD were determined by PCR

Intestinal biopsy was recommended to subjects testing positive to both the serologic and the genetic tests, so as to obtain definitive diagnosis of CD.

- At the end of the study we find that **Nine subjects** (9/120, **7.5%**, 6 M, 3 F) with severe food allergy tested positive for both the serological CD markers (AEA and anti-tTG), and HLA DQ2/8 haplotypes.

The results obtained so far reinforce the idea that **active screening of celiac disease is necessary among those with severe allergy and that this practice should be included in the pediatrician clinical activity.**



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

Pilot project “Detecting and managing CD patient within a 'cohort of super allergic population”

Start-up description template



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 (CD) is a systemic gluten-dependent autoimmune disease often occurring in association with other autoimmune disorders.
- **The relationship between CD and allergy is still unclear**, but some reports suggest that patients with celiac disease have an increased frequency of allergic manifestations compared to general population.
- One factor that might trigger allergies in celiac patients is abnormal intestinal permeability which increases the flow of dietary antigenic protein, predisposing the mucosal immune system toward an atopic condition.
- Moreover, CD and allergy might share a similar predisposing genetic background.

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

- **Experience of one of the partners with ELISA diagnostic test in population screening**
- Experience of one of the partner with Allergic patients.
- **Preliminary screening of Super allergic patients for the discovery of CD patients**

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

- Several studies have addressed the frequency of allergic manifestation in CD population
- Williams A. *Coeliac disease and allergic manifestations*. Lancet 1987;1:808.
- Kelly C, O'Shea B, Kelly J, Feighery C, Weir D. *Atopy and childhood coeliac disease*. Lancet 1987;2:109.



- Verkasalo M, Tiilikainen A, Kuitunen P, Savilathi E, Backmann A. *HLA antigens and atopy in children with coeliac disease*. Gut 1983;1129:306-310
 - *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 in PP7 Burlo in collaboration with allergy department to collect serum samples of Allergic patients**
- **we plan to use existing infrastructure in PP7 Burlo in collaboration with gastroenterology department to collect tissue samples when biopsy is performed.**
- **All serum samples will be transported with portable freezer to partner PP5 Units location in Trieste.**
- **Serum will be tested By PP5 UNITS**
- **Tissue samples will be stored in liquid nitrogen containers for further analysis.**
- **If results are confirming hypothesis of increased frequency of CD within this population we will try to publish results into high IF journals as present it at conferences.**



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...)*
 - The aim of this study is to evaluate the prevalence of celiac disease in children with very severe food allergy, undergoing specific food oral immunotherapy (OIT).
 - In addition, information regarding clinical symptoms, laboratory data, and OIT outcome will be collected about the newly diagnosed celiac patients both before and after diagnosis, so as to investigate whether the gluten-free diet (GFD) had any effect on the allergic manifestations.

- *What is the approach that makes the project viable and sustainable?*
 - We have preliminary data confirming that super allergic patients have an increase frequency of CD
 - By putting together different expertises such as allergologists, GI and biotechnologist will speed up patients' collection and analysis
 - Role of one the partner as a reference centre for coeliac disease will enable partner to provide service for other institutions in the region.
 - Role of one the partner as a reference centre for allergy in the region will enable partner to provide service for other institutions in the region.

- *What kind of problems are you anticipating and what is your "plan B"-s if something doesn't turn out as you counted in certain situations?*
 - Inability to collect enough samples is possible, however given the typical rate of patients seen in the hospital make this risk very small.



Will the pilot have cross-regional impacts? Which are they?



- this study will identify a new clinical condition at risk of CD, which calls for screening in other hospitals within the region and to other health care institutions in order to prevent possible complications of untreated CD.
- We will present our experience at regional, national and international meetings to other potential stakeholders as well as through the scientific publication we will prepare.
 - Any other aspects you find important?
- Discovering new way to find CD patients in at risk or even asymptomatic population is always a driving force for a better understanding of the disease.



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>
1. Biology/immunology experts	Research professionalsD	design and implementation of pilot project's main activities	improved knowledge of the molecular bases of the disease
2. Medical experts - specialists gastroenterologists, pathologists	Healthcare professionals	Co-operation with diagnostic activities	Improved diagnostic possibilities, better service
3. Medical experts - Allergologists	Healthcare professionals	Provide patients samples and clinical data	Improved diagnostic possibilities, better service



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

<p>Key pilot Partners</p> <ul style="list-style-type: none"> • Project partners (PP5 UNITS-PP7 IRCCS Burlo) • Medical experts (specialists gastroenterologists, allergologists) 	<p>Key Activities</p> <ol style="list-style-type: none"> 1. Sampling of Serum. 2. Testing of serum 3. Sample of tissue 4. Clinical and diagnostic data analysis and correlation <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"> • PP5 UNITS • PP7 BURLO 	<p>Value Proposition of the pilot (what is the benefit?)</p> <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. 	<p>End-user Relationships</p> <ul style="list-style-type: none"> • Partner will be able to provide better service to end users (patients) <p>Communication channels?</p> <ul style="list-style-type: none"> • personal contacts • web based communication 	<p>End-user Segments</p> <p>Over 200 Allergic patients tested and approximately 3-5 new CD patients identified</p>
<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. serum sample collection	Project partner Burlo	200 samples frozen	October 2018
2. test of serum samples for TG2 antibodies	Project partner UNITS	Anti TG2 and EMA test performed	November 2018
3. positive patients recall genetic test and and Biopsy taken	Project partner Burlo + UNITS	5-10 biopsy samples frozen	November 2018
4. Biopsy analysed	Project partner UNITS and BURLO	Detection of positive samples Genetic test	January 2019
5. Statistical analysis of the collected data	Healthcare professionals specialist in <i>medical biochemistry</i>	Getting the appropriate and quality data	January /2019
8. Comparison of results with the current knowledge	Project partners, medical experts	Improvement of the quality of the patient's care	January /2019
9. Presentation/spreading the data	Project partners, medical experts	Improvement of the general knowledge	January /2019

ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

Pilot

“Detecting and managing CD patient within a 'cohort of super allergic population” PP5 UNITS

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 evaluate the prevalence of celiac disease in children with very severe food allergy, undergoing specific food oral immunotherapy (OIT).
- In addition, information regarding clinical symptoms, laboratory data, and OIT outcome will be collected about the newly diagnosed celiac patients both before and after diagnosis, to investigate whether the gluten-free diet (GFD) had any effect on the allergic manifestations

- **The approach that makes the project viable and sustainable:**

- We have preliminary data confirming that super allergic patients have an increase frequency of CD
- By putting together different expertise such as allergologists, GI and biotechnologist will speed up patients' collection and analysis
- Role of one the partner as a reference centre for coeliac disease will enable partner to provide service for other institutions in the region.
- Role of one the partner as a reference centre for allergy in the region 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
- PP7 Allergy Department and Immuno-diagnostic Department
- FVG region GRAP (Pediatric allergologists working group)
- Novara, IPAZIA Center for the study of autoimmune disorders.

We have organized several meetings:

- On February 2018 in Novara **“FOCUS IN CD dissemination”**

The Stakeholder meeting was attended by Biologist and Doctors that are member of the research centre for autoimmunity in Novara. The WPT3 pilot project related to the detection of CD in super allergic population was presented and described. Collaborations for sample and other material exchange were established. A visit to the new IPAZIA research building has also take place.

- 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.

- PP5 organized together with the PP7 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 screening of celiac disease among children with severe allergy to food proteins.

From the practical point of view The PP5 have enrolled 75 subjects with severe food allergy and identified 4 subjects with celiac disease following the ESPGHAN criteria. Among these children the incidence of celiac disease was 5.3%.

In addition, we enrolled 50 children with moderate food allergy who spontaneously acquired food tolerance and none tested positive for celiac disease serological markers.

All the biological samples are undergoing to the analysis, as well as the follow-up of the new CD-patients.

With these results we discussed with pediatric allergologists of our region to adopt a common guideline that includes celiac screening among children suffering from severe food allergy.

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.T3.2.6 “Detecting and managing CD patient within a 'cohort of super allergic population”

Pilot project - 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.

We have preliminary data confirming that super allergic patients have an increase frequency of CD, By putting together different expertise such as allergologists, GI and biotechnologist our aim was to speed up patients' collection and analysis to confirm data. This study had the aim to identify a new clinical condition at risk of CD, which calls for screening in other hospitals within the region and to other health care institutions in order to prevent possible complications of untreated CD.

Our Pilot project “Detecting and managing CD patient within a 'cohort of super allergic population” comprises 3 topics:

1- Evaluate the prevalence of celiac disease in children with very severe food allergy, undergoing specific food oral immunotherapy (OIT).

Patients were included in our study according to the following criteria: elevated IgE levels against food proteins (IgE values > 85 kU/l) and a positive history of at least one severe allergic reaction (i.e., a reaction defined as classes 4 and 5 according to Clark's classification) after accidental or voluntary (oral challenge) exposure to the causal food.

One the partner, as a reference centre for allergy in the region, has collaborated to identify patients and provide samples for screenig.

During the period of activity of the pilot project we have enrolled a total of one hundred and twenty children (72 M, 48 F, mean age 10±3 years) with very severe food allergy (98 with cow's milk allergy, 48 with egg allergy, 15 with wheat allergy).

Serum samples were analyzed

- for IgA anti-endomysium antibodies (AEA) by indirect immunofluorescence on cryostat sections of human umbilical cord,
- IgA-IgG anti-TG2 antibodies were measured using an ELISA (Eurospital, Trieste, Italy) following the manufacturer's instructions (normal values <7 U/l).
- The susceptibility alleles for CD were determined by PCR with allele-specific primers identifying HLA DQ2 and DQ8, using Eu-Gene-Risk kit (Eurospital).

Intestinal biopsy was recommended to subjects testing positive to both the serologic and the genetic tests, so as to obtain definitive diagnosis of CD.



Endoscopy was done and small bowel biopsies showed mucosal intestinal atrophy. All patients were placed on GFD.

The immune assays and biopsy analysis were performed by operators blinded to the subjects' clinical and laboratory data.

At the end of the study we find that Nine subjects (9/120, 7.5%, 6 M, 3 F) with severe food allergy tested positive for both the serological CD markers (AEA and anti-tTG), and HLA DQ2/8 haplotypes.

The results obtained so far reinforce the idea that active screening of celiac disease is necessary among those with severe allergy and that this practice should be included in the pediatrician clinical activity.

2 Collection of information regarding clinical symptoms, laboratory data, and OIT outcome from the newly diagnosed celiac patients both before and after diagnosis, to investigate whether the gluten-free diet (GFD) had any effect on the allergic manifestations.

Clinical and laboratory data were collected regarding the newly diagnosed cases of CD, and eventual changes before and after GFD were correlated.

The prevalence in the study group was compared with that of 128 age-matched children (68 M, 60 F, mean age 11 ± 2 years) who had made a spontaneous recovery from food allergy without OIT and also with a control group made up of 3188 healthy schoolchildren screened for CD in Trieste, in these last two groups the prevalence was equal to 1%.

Specific IgE, IgG4 and the tolerated dose were measured before and after OIT and no differences were observed between patients with only severe food allergy and patients with both severe food allergy and CD.

More specifically, with regard to children allergic to cow's milk, in eight untreated CD patients (before diagnosis), four treated CD patients (on GFD for more than 12 months), and 40 non-celiac allergic patients, we measured the minimum trigger dose to induce severe symptoms before oral immunotherapy and the maximum tolerated milk dose 10 days after oral immunotherapy. No statistical differences were found among these three groups regarding the cow's milk-tolerated doses, with no differences among untreated and treated CD patients ($P = 0.8$).

3) Sharing of the knowledge of severe allergy and CD clinical connection among HCP in northeastern Italy.

- Results: we have organized several scientific meetings where we have illustrated both the general idea of the project FOCUS in CD as well as the pilot project for the diagnosis of CD in super allergic population. More significant were **two meetings with over 500 pediatricians and family doctors from our region (Grado 11 October 2018) and Veneto (Treviso 16 November 2018)** dedicated to improving the knowledge regarding the diagnosis and follow-up of celiac disease.



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
<p>1. We have identified a new group at risk of celiac disease and diagnosed celiac patients that were unaware of their disease condition. Our screening will help them to better manage their condition, to gain knowledge of the disease, to reduce problems and disorders complications risks thanks to a strict diet compliance.</p>	<p>1. We have confirmed that a specific population of patients (super allergic) have a higher risk of presenting as associated disorder CD. This will lead, by the help of HCPs and stakeholders, to share new CD-diagnostic guidelines.</p>
<p>2. We have shared our knowledge on the allergy/CD relation with pediatricians and allergologists of the region that are now able to recognize and manage these new CD related clinical conditions.</p>	<p>2. We have established a stable presence of stakeholder groups on this specific field that will allow the diffusion of the knowledges acquired during this project and at the end better quality of life of allergic/celiac disease patients</p>

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 successfully reached all intended outcomes of our pilot. Collaborations within the two projects partners involved help to achieve all tasks planned.

The only relative problem encountered during the project was due to possibility of accessing very large numbers and diversity of samples to be tested. This limit as to be seen in terms of:

- absolute numbers of patients tested. During the pilot we limit the number of patients by working only with the one accessing directly to the PP7 hospital clinical allergy service



- limited variability of the patients selected especially in term of ethnic origins.

These limits, now that we have positively validated the model could be easily overcome by a multicentre/multinational study.

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. The merge of basic research and daily clinical practice (both in the clinical allergy service as well as in the gastroenterology one) has force both partners to have frequent interactions and share mutual feedback on clinical results and patient's follow-up. This has strengthened a stable collaboration that has substantial advantages for both the patients as well as for all the staff involved.	1. There were no specific setbacks in our project
2. The close collaboration within the stakeholders ie the regional allergological group, the center for research in autoimmunity (in Novara -Italy) and PP5 and PP7 has created a scientific network with wider connections (national and internationals) that will help in disseminating out the outcomes and guidelines produced.	2.

5. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

- What are your further activities of the pilot project development,
 - > On the local level ?
 - > On transnational level ?

On the local level ?



Our main aim is to maintain strong interactions within the stakeholder group that is constituted by pediatricians, allergologist, gastroenterologists and applied biology/immunology researcher.

We plan to have one or two annual meetings within the discussion group to share clinical cases and share innovations on CD-diagnostic methods.

On transnational level ?

we aim to present our data that link severe allergy and celiac disease to colleagues both in other “working groups” as well as in scientific societies working in the same field (CD and Allergy) to increase awareness and knowledge about our pilot project discovery. Data from this study will be presented at the ESPGHAN annual meeting in Glasgow 5-8 June 2019 as a poster presentation (A-1071-0003-01001).

- 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?
- Sustainability:
 - the idea behind the pilot project (find a link between sever allergy and CD) was verified by a working group (paediatricians, allergologists, gastroenterologists and researcher) that was born during the project, but that as single units continue to operate continuously even after the end of the project. Therefore, no problems are foreseen to continue a future collaboration in this specific field which is part of the normal therapeutic diagnostic institutional activities.
 - The availability of Guidelines acquired by the pilot clinical and diagnostic experience will allow to diffuse the knowledge and the application of the screening procedure for CD diagnosis by other working groups so to become a hopefully standard diffuse procedure.
 - Sustainability can be foster by application to other competitive research grants in the future
- Transferability and cooperation:
 - *Deliverable D.T3.3.1 “Transnational transferability plan of pilot solutions” -*
 - *Deliverable D.T3.3.2 “Pilot project recommendations for transfer to other users/regions”*
 - Based on feedback from pilot stakeholder and by using our clinical and scientific network with plan to transfer our diagnostic protocol to other possible users within the consortium participating regions as well as to extra projects regions.*
 - *e-tools will be promoted as well.*