

Proceedings

COELIAC DISEASE (CD) AND NON-HODGKIN LYMPHOMA (NHL)

5th BIOMED MEETING

June 29-30, 2001

**Dublin, Fitzpatrick's Castle Hotel
Ireland**

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Guests from Dublin: Dr. N. Kennedy, Dublin (Nutritionist), Dr. Sean O'Brian, Dublin (Histopathologist), Ms. S. Wilson (MSc Student, Australia)

Guest speakers C. Coates BSc (St. James's Hospital, Dublin), C. O'Farrelly PhD (Dir. of Educational & Research Centre, St. Vincent's Univ. Hospital, Dublin), Dr. K. Kaukinen (Tampere), C. Kilmartin PhD student, (St. James's Hospital, Dublin), Dr. C.J.J. Mulder (Arnhem) and Drs. J.J. Schweizer (Leiden).

Friday June 29, 2001

14.00-14.30

Opening remarks

C. Feighery:

Welcome in Dublin. I hope you will enjoy your stay in this here. Unfortunately Geoffrey Holmes can not attend this meeting due to a hip problem. We will miss him very much and hope that he will soon recover, as he is one of the cornerstones of our group. In honour of Geoff we should strive to have as good a meeting and try to be as conclusive as we can in whatever we achieve.

This is very informal Dublin, Ireland. The people that you will meet here have 3 names: Mo (Mohamed Abuzakouk), Con (C. Feighery) and Teresa Shaw colloquially known as T.

Agreements

The Hague Meeting:

M.L. Mearin:

1. As stated in Alghero: if a partner wishes to report the staging of the NHL it should be done consistently for all the cases.

Italy (102), Sweden (104), Finland (105), Belfast (106), Galway (206), Barcelona (208), France (109) and Poland (110) report the staging.

2. All the partners should send biopsy samples of NHL-cases with CD to N. Brousse in Paris for intraepithelial lymphocytes quantification as soon as possible.

DNA for HLA typing of all NHL-cases with CD should be sent to J. Schweizer in Leiden as soon as possible.

It will be impossible to send the samples of some patients.

3. As for the assessment of the GFD, G. Holmes will inform Leiden about his protocol.

You have received a questionnaire according to the protocol of G. Holmes. We will discuss later how to integrate that.

4. Data of all consecutive NHL-cases and controls should be in Leiden by 15th April 2001. Any case arriving later will be refused. Final data about those cases and controls should be in Leiden by 15th September 2001. No additional information is accepted after that date.

This has been well accomplished, but some cases may have to be taken out when we carefully check the database.

5. The date set for the 5th Biomed Progress Meeting in Barcelona is 11th and 12th May 2001.

The meeting in Barcelona has not taken place. In its place is this meeting in Dublin.

6. The final working meeting will be held in November 2001. Dublin is tentatively considered as the place where it will take place.

Tomorrow we will discuss a location for the 6th working meeting. During the meeting in The Hague it was not yet known that an extension of the project would be given until 31st December 2001. So we will have to plan the time between now and then.

7. For the very final meeting there are two options:
a. attached to the Coeliac symposium that will be held in Paris in June 2002. Half a day to one day would be sufficient;
b. sponsored through the EC and maybe in Brussels.

I am glad that J. Schmitz could be here so that we can discuss that tomorrow.

8. The approval by the Medical Ethics Committees from, Derby, London, Galway, Belfast and Madrid for cases and/or controls should be sent to Leiden as soon as possible.

For publishing the data it will be important to have the MEC. Galway will send it by postal mail. Derby should send it too.

**Partners' Presentations:
Present position
Cases and Controls**

Chair: C. Feighery

Cases:

101 The Netherlands

J. Schweizer, Leiden

Since I work both in centres and in an area I will combine the presentation. I work in one area, which is defined by postal code and is one of the regions of our cancer registry.

• Comprehensive Cancer Centre West (I.K.W)

Hospitals	12
Pathology laboratories	9
Inhabitants > 18 years	2.35 x 10 ⁶

• Three Centres:

LUMC (Leiden University Medical Centre)
AMC (Amsterdam Medical Centre)
RHA (Rijnstate Hospital Arnhem)

• Research period

Starting date

IKW	1998-11-01
RHA	1999-01-01
AMC	2000-01-01

Closing date 2001-02-28

Identified cases in both area and centres: 770

<u>Invited cases:</u>	452	= 58.7%
inf. consent:	223	= 29.0%
no inf. consent:	157	= 20.4%
no response	72	= 9.4%

<u>Not invited:</u>	318	= 41.3%
deceased:	81	= 10.5%
not asked:	198	= 25.7%
missing:	39	= 5.1%

There were several reasons for “not asked” E.g. the physicians in charge thought the patients were not fit enough to participate, or because of language problems (foreigners).

“Missing” refers to data that is not yet in the database, but it will be by September 15, 2001. The majority of the 223 cases (about 50% of the invited cases), came from the region of the I.K.W.

The mean participation rate is about 30%.

Sex distribution:

	<u>YES</u>	<u>NO</u>	<u>DIED</u>	<u>NOT INVITED</u>
M	123	75	40	140
F	99	82	41	129
M/F	1.2	0.9	1	1.1

Age distribution:

	<u>YES</u>	<u>NO</u>	<u>DIED</u>	<u>NOT INVITED</u>
M	60.3	69.4	67.7	62.9
F	58.5	69.9	71.1	68.7

As you see the participants are younger than the non-participants.

NHL most frequent diagnoses:

N= 682

unknown 88 (11.4%)

B cell = 609 (89.3%)

T cell = 73 (10.7%)

dif.large3	= 254	an-LTL	= 16
follicular centre	= 145	mycosis fungoides	= 10
lymoplasm acytoid	= 62	peripheral T cell	= 9
B-CLL/SLL	= 60	T-cell, Nos	= 7
mantle cell	= 34	EATL	= 5

The large number of rare mycosis fungoides is due to a new professor of dermatology who came to the LUMC half way through the study and who happens to be the national expert on cutaneous lymphomas.

Primary site (most frequent):

	<u>ALL</u>	<u>YES</u>	<u>NO</u>	<u>DIED</u>	<u>NOT INV.</u>
1. nodal	nodal	nodal	nodal	nodal	nodal
2. bone marrow	BM	BM	BM	s.bowel	BM
3. skin	stomach	stomach	skin	BM	stomach

The small bowel was the second most frequent site for the patients who died. Skin was third but that was because of this new professor.

Primary site small bowel

	<u>ALL</u>	<u>YES</u>	<u>NO</u>	<u>DIED</u>	<u>NOT INV.</u>
total n.	19	3	3	9	4
REAL	5x23	2x23	3x23	1x28	1x28

23 = EATL.

28 = peripheral T cell lymphoma

The rest were B cell lymphomas.

Summary CD investigations

CD prior to study:	4 (3 refractory)
CD through study	3
all	7/223
IKW	3/180
AMC	0/12
RZA	1/31 or 4/35

- Controls of The Netherlands:** The control group formed by 1440 person, picked at random, comes from 2 studies:
1. The Dutch project on coronary heart disease and nutritional habits.
 2. MORGEN: the biggest population based study in the world on food and cancer, which included 400.000 people, 30.000 from the Netherlands.
- Equal sex distribution
 - Age range 20-59 years

Plans until 15th September 2001

- Work on remaining cases
- Re-invite “not asked” from January 2000 onwards
- Check data for accuracy
- Collect missing data
- Complete control group
- As a local project we will review all the 19 small bowel lymphomas

Discussion:

L. Mearin:

You say that you have studied 223 cases with NHL?

J. Schweizer

I got informed consent from 223 cases, but I have until this moment the results of 180. Most of them have already been screened. The difference will vanish as soon as the results will be known.

C. Catassi:

1. We should discuss whether the 3 cases of refractory CD should be taken out.
2. Will you be able to biopsy the controls?

J. Schweizer:

To start with the second question, I am not sure yet. I may get about 6 or 7 positive screenings and for those I will ask permission for a biopsy. I was bound to fail if I had asked permission before the screening. But I will be able to get DNA from them.

As for the first question, I agree.

C. Mulder:

I can accept that we refuse those 3 refractory cases but then we have to discuss them in the results and say why we do not include them.

L. Mearin:

It is a difficult decision. I think we should keep them because they are coeliac patients who got a NHL during the period of our study, even if they have a special form of CD being refractory coeliacs.

C. Hallert:

How many were EMA positive?

C. Mulder:

Their small bowel biopsies were compatible with CD.

L. Mearin:

We agreed to work symmetrically in cases and controls. We found recognised coeliacs, with no need to be screened, and unrecognised coeliacs found through screening. Those 3 refractory cases were recognised coeliacs.

J. Schweizer:

They might have had a lymphoma for 10 years but this was only diagnosed during the period of our study. However, we might treat them in a special way because it also gives extra information about what kind of patients we recruited.

C. Catassi: So there may be two estimates of frequency of CD in NHL with and without them.

In the Italian study we did not include refractory CD, but there are arguments as well to include them.

C. Feighery:

We will probably end up analysing them both ways and then see what effect they have by inclusion or exclusion.

Cases:**102 Italy****E. Fabiani, Ancona**

We collected in a Multicentre study in Italy in 10 centres, widely spread throughout the country, all adult (> 18 years of age) consecutive NHL-cases. These cases were of any kind and of any primary site. They were collected in each of the centres either in the clinical medicine department, in the haematology, or oncology department. We did not investigate any lymphoma within a specific area.

Every centre has its own UIC (unique identification code).

The Italian participating centres:

Centre	NHLs (no.)
102.0--- Ancona_	31
102.1--- Aviano	14
102.2--- Bari	2
102.3--- Naples	49
102.4--- Padua	2
102.5--- Palermo	18
102.6--- Rome	23
102.7--- S. G. Rot.(FG)	69
102.8--- Verona	18
102.9--- Modena	18
Total	244

Of the 10 hospitals involved 7 were university hospitals.

There were also 2 Gastroenterology centres involved, one from Naples and one from Modena.

The study period was from May 1998 until 31st of March 2001.

Some centres stopped before collecting cases because of practical problems. See following table.

The overall percentage of participation was 32% in 1998, 28% in 1999, 22% in 2000 and around 15% in 2001. In the Italian study (3038 NHL cases and 1253 participants) the percentage of participation was 27%

Centre	5/98-12/98			1999			2000			1/01-3/01		
	E	%	O	E	%	O	E	%	O	E	%	O
Ancona	11	26.8	41	14	35	40	7	40	17	0	10	0
Aviano	12	41.4	29	1	2	50	0	0	50	4	33	12
Bari	0	0	40	2	2.5	80	stop collecting NHL				
Modena	9	47.4	19	26	83.9	31	20	50	40	1	10	10
Naples	30	85.7	35	29	67.4	43	7	16.3	43	1	10	10
Padua	2		?	stop collecting NHLs				
Palermo	3	10	30	20	50	40	14	50	28	2	28.6	7
Rome	17	22	77	6	4.7	127	stop collecting NHLs				
S.G. Rotondo	27	38.6	70	37	52.8	70	8	11.4	70	9	52.9	70
Verona	8	26.6	30	9	30	30	2	6.7	7	0	0	7
Centres	5/98-12/98			1999			2000			1/01-3/01		
	E	%	O	E	%	O	E	%	O	E	%	O
Overall	119	32	371	144	28.2	511	58	22.7	255	17	14.6	116

Enrolled/studied NHLs percentage in each Italian participating Centre during the study period.

E (no.)=number of enrolled NHLs;

O (no.) = number of studied NHLs

% E NHLs (n)=338/ O NHLs (n=1253) during the whole study period.

The overall NHLs

No.	338
Females	144 (43%)
Males	194 (57%)

Mean age females	57
Age range females	19 – 92
Mean age males	57
Age range males	21 - 92

There are 14 cases, which are still being reviewed by the pathologists.

NHL primary Site

Lymph nodes	147
Stomach	30
Mediastinum	28
Bone	17
Spleen	14
Skin	13
Tonsils	11
Small bowel	8
Waldeyer's ring	7
Retroperitoneum	5
Bone marrow	4
Lung	4
Brain	4
Testis	3
Salivary glands	3
Mouth	3
Liver	3
Colon	2
Soft tissue	2
Thyroid	2
Uterus	2
Other	15
Unknown	11

Gut (overall) 12% of all NHL

NHL histology (REAL)

Diffuse large B-cell lymphoma	148
Follicular centre lymphoma, follicular	38
Extranodal marginal zone B-cell lymphoma	18
Mantle cell lymphoma	17
High-grade B-cell lymphoma, Burkitt-like	16
Lymphoplasmacytic lymphoma	16
Follicular centre lymphoma, diffuse, small-cell	6
Peripheral lymphoma, unspecified	6
B-cell NOS	6
Nodal marginal zone B-cell lymphoma	4
Angioimmunoblastic T-cell lymphoma	4
Sezary syndrome	3
Primary mediastinal large B-cell lymphoma	2
Splenic marginal B-cell lymphoma	2
Anaplastic large cell lymphoma, T-cell type	2
Mycosis fungoides	2
Hairy cell leukaemia	1
Anaplastic large cell lymphoma, null-cell type	1
Intestinal T-cell lymphoma without enteropathy	1
Angiocentric lymphoma	1
Unknown	44

There are still 57 sera to be tested: 15 from Naples and 42 from Modena. In this last centre they can not test for EMA, but only for antigliadin. We will have to decide what to do with these sera.

L. Mearin:

You can send the sera to Holland and the EMA test will be performed in Amsterdam.

C. Catassi:

There was not one positive screening in Modena?

E. Fabiani:

No it was negative because he was on a gluten free diet during the study: he was a case of recognised CD prior to the study.

Controls of Italy:

The controls: size and source

No. = 3282 subjects
General population screening (Campogalliano, Modena)

The Dionysos Project (Campogalliano, Modena)

Project starting date: 1992

Serology testing (EMA) 1999

Jejunal biopsy (upper GI endoscopy) 1999

CD diagnosis 1999

Volta U, et al.

High prevalence of coeliac disease in the Italian general population.

Dig Dis Sci 2001 (in press)

No. 3382
Females 1707 (52%)
Males 1555 (48%)
Age range 9 – 70

EMA test results:

Positive EMA 20/3282 (7 M, 13 F)

1 female and 1 male were recognised CD cases and the other 18 were discovered through the screening. Of all the 18 cases found through the study 16 were biopsy proven CD. The other 2 who were EMA positive but histologically not confirmed to be coeliacs.

EMA positive case of the Control group

Control	Sex	Date of birth	CD Prior	Confirmed by biopsy	EMA+	After EMA+: biopsy performed	CD established after screening
1	F	1929/08/06	no	yes	yes	yes	yes
2	F	1974/08/13	no	yes	yes	yes	yes
3	F	1975/02/27	no	yes	yes	yes	yes
4	F	1977/12/02	no	yes	yes	yes	yes
5	M	1940/02/01	no	yes	yes	yes	yes
6	M	1931/05/07	no	yes	yes	yes	yes
7	F	1979/09/12	yes	yes	yes	yes	yes
8	M	1970/02/06	yes	yes	yes	yes	yes
9	F	1936/05/13	no	no	yes	yes	yes
10	M	1975/12/05	no	no	yes	yes	no
11	F	1963/12/26	no	yes	yes	yes	yes
12	F	1972/03/16	no	yes	yes	yes	yes
13	M	1966/10/28	no	yes	yes	yes	yes
14	F	1958/08/25	no	yes	yes	yes	yes
15	F	1972/07/27	no	yes	yes	yes	yes
16*	F	1950/11/25	no	no	yes	yes	no
17	F	1976/04/24	no	yes	yes	yes	yes
18	M	1974/01/21	no	yes	yes	yes	yes
19	M	1958/04/11	no	yes	yes	yes	yes
20	F	1942/12/08	no	yes	yes	yes	yes

J. Reehuis:

Number 9 in your table: it states 'no' for 'confirmed by biopsy', but 'yes' for 'CD established after the screening'.

E. Fabiani:

I will ask to clarify the information about this control.

L. Mearin:

What should we do with those centres that stopped collecting before the end of the project?

C. Catassi:

They started before 1998 and stopped after 3 years.

E. Fabiani:

It will be clearly described when each centre stopped collecting cases.

C. Feighery:

It would be interesting to get more information on these 2 controls who were EMA positive but histologically not confirmed to be coeliacs, for example IEL etc.

E. Fabiani:

I can ask whether there is some DNA available or some DQ determinations done, or if it is possible to collect some blood from them to type their HLA.

C. Feighery:

The simplest thing is to have the biopsy reviewed, as there is biopsy material, and if by repeating the tests the EMA are still positive.

E. Fabiani:

I will move in that direction.

Cases:

103 United Kingdom

Derby

L. Mearin:

The total number of cases for Derby are:

101 NHL cases

2 cases of CD: 1 prior to the study and 1 found through the study and biopsy confirmed.

Controls of Derby, UK:

The 457 controls are diabetes type II. It has already been published (Page, et al), showing that the frequency of CD is not increased in diabetes type II.

Cases:

203 London

P. Ciclitira

J. Fraser can not be here but she has collected 24 NHL-cases. Our problem is that we could not get the approval by the Medical Ethical Committee to work with other centres. So we are limited to our local area. The haematology registry was very unhelpful as well. J. Fraser had to sit in the clinic herself every week to pick up these cases. It was very labour intensive and that is why we got such a small number. I do not see that number increasing.

Cases:

106 Ireland

Dublin, M. Abuzakouk

Area: one institution: St. James's Hospital

All consecutive cases of NHL patients

Study commencement date: 22/12/1998

Study Closure date: 10/04/2001

Number of cases collected: 72

Males:

Number of cases: 31

Mean age: 60 year (range 22 – 90)

Females:

Number of cases: 41

Mean age: 64 year (range 32 – 85)

Results of IgA, EMA and AGA:

IgA (62/72): no deficiency detected

EMA (56/72): one positive

AGA (56/72): 3 marginally positive (5.2, 5.3, 7.5)

Normal range (0 – 5 AU)

Of the 72 patients we found 3 patients with gluten sensitive enteropathy.

1. Dermatitis herpetiformis (DH)

1977, GFD, well at present

2. Coeliac disease:
1995, GFD, RIP
3. Coeliac disease:
2000, GFD x 3 months, RIP

The primary site:

Lymph node	26	Liver	1	Mediastinal mass	1
Bone marrow	7	Gum	1	Chest wall mass	1
Tonsils	5	Lung	1	Retroperitoneal mass	1
Stomach	5	Omentum	1	Unknown	2
Spleen	5	Pharynx	1		
Intestine	2	Nose	1		
Parotid	2	Caesum	1		
Testis	2	Skin	1		
Thyroid	2	Thymus	1		
Bone	1	Mouth	1		

Type of lymphoma REAL classification

B cell:	56 (78%)	T cell:	4 (5%)
Lymphoplasmocytic:	3 (4%)	Follicular:	2 (2%)
Marginal zone:	1 (1%)	MALT:	1 (1%)
Mixed cell	1 (1%)	Undetermined	4 (5%)

Staging: not provided

Controls of Ireland:

The MONICA study that P. Watson presented before forms the control group.

All MONICA studies are constructed in the same way. 5000 people between the ages of 25-65. 1500 in cohorts of 10 years. 25-35, 35-45 and so forth took part, half males and half females. P. Watson had access to 1823 sera from the 1991 MONICA study in Northern Ireland and determined EMA, AGA and anti-reticular antibodies. He has adapted the results for our Biomed study by EMA positivity. There were 22 individuals detected with CD: 2 known coeliacs. 1:83. There were 3 people who gave no consent. 17 were asked for biopsy and 10 agreed: 9 had CD and 1 was negative. 2 known +9 discovered = 11:1823=1:166

The data in terms of age distribution:

age & sex	nr.of controls	EMA	SBB	Result	CD established now	CDprior
				Enteropathy+	enteropathy + and CD prior	
	participants n=1823	n=22	n=10	n=9	n=11	n=2
25-29M	69	1				
25-29F	77					
30-34M	105					
30-34F	103	1				
35-39M	94	1		1	1	
35-39F	100					
40-44M	97	2				
40-44F	112					
45-49M	146	2		1	1	
45-49F	120	1				
50-54M	143	2				
50-54F	124	0				
55-59M	137	4		1	1	
55-59F	132	6		3	3	
60-64M	142	1			1	1
60-64F	122	2		2	2	
65-69M						
65-69F				1	2	1
Total	1823	22		9	11	2

Cases:

109 France

N. Bousse, Paris

Recruitment of lymphoma cases: 74.

One centre: The haematology department of Necker-Enfants Malades – University Hospital in Paris (Professor Bruno Varet)

All new consecutive cases, with lymphoma, of all kinds, from January 1999 until 14th April 2001 were recruited.

85 cases were invited to participate: 74 gave consent (response 87%)

74 recruited cases:

Mean age: Male = 58 (18 – 91) female = 56 (20 – 90)

Number of male = 45 / female = 29

Stage: I = 14 (18%); II = 9 (12%); III = 11 (18%); IV = 40 (54%)

No positive anti-endomysium antibody

No CD prior to entering the study

Diagnosis of 74 lymphomas according to REAL classification

2 = B-CLL /small lymphocytic L.	5,4%
4 = diffuse large B-cell L.	52,7%
6 = follicular L.	13,5%
7 = follicular, diffuse small cell L.	1,35%
9 = high grade B-cell, Burkitt-like	4%
11= Mantle cell L.	13,5%
15= mediastinal large B-cell L.	2,7%
20= angioimmunoblastic T-cell L.	2,7%
21= hepatosplenic gamma-delta T-cell	1,35%
28= peripheral T-cell L.	1,35%
29= precursor T-lymphoblastic L.	1,35%

Ch. Mulder:

The hepatosplenic gamma/delta T cell lymphoma is quite an interesting one. Do you have small bowel biopsies of that patient?

N. Bousse:

No we have the bone marrow and the liver biopsy.

We did not include our cases with refractory CD, because we are a reference centre for that in France and these cases with refractory CD were diagnosed elsewhere.

Controls of France:

They come from the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) study. Among the 493 controls tested for EMA there were no positive ones. In total there will be more than 4000, but they will not be tested for EMA.

Number of male = 236 (48%) / female = 257 (52%)

Mean age: Male = 53.6 – Female = 57.6

No EMA positive

No previous CD

Discussion:

J. Reehuis:

I think there is one coeliac in the control group.

N. Brousse:

One control was a known coeliac on a gluten free diet and negative EMA test.

C. Feighery:

You found one coeliac in the control group and no coeliacs in the NHL-cases.

Ch. Mulder:

If you have several centres you can expect that one centre will have a very low incidence of CD.

L. Mearin:

How did you choose those 493 controls?

N. Brousse:

At random.

M. Abuzakouk:

Did you test the total IgA in serum to establish IgA deficiency?

N. Brousse:

Yes, but I do not have the data here.

J. Schweizer:

Maybe there are biases as the controls are quite healthy.

J. Schmitz:

We discussed it several times and we thought they would not be healthier than the blood donors. These people are probably healthier than the general population, but not healthier than other control groups are.

J. Schweizer:

The people who are rejected as blood donor have more CD than the ones accepted as donors have.

L. Mearin:

Anaemia, which is a reason not to accept someone as a blood donor, can be a symptom of CD.

Cases:

110 Poland

Hania Szajewska, Warsaw

The data from Poland all comes from one centre, which is the Institute of Oncology in Warsaw.

Starting date: 20th April 1999

Final date: 9th April 2001

Number of cases eligible for enrolment: 99

Number of cases enrolled: 86

Recruitment rate: 87.5%

Male / Female 55 / 44

Mean age males 58.2 (18.9 – 84.9)

Mean age females 58.5 (17.6 – 80.2)

As in other centres the most common primary site is lymph nodes.

Primary site

2. Bone	1%
3. Bone marrow	5.1%
5. Colon	1%
8. Lung	10.1%
9. Lymph nodes	44.4%
11. Spleen	2%
14. Stomach	9.1%
16. Testis	3%
19. Salivary glands	2%
20. Skin	1%
21. Small bowel	1%
26. Other	20.2%

Histology

2. B-CLL/SLL	12	12.1%
3. Burkitt	2	2%
4. Diffuse	20-	20.2%
5. Extranodal	4	4%
6. Follicular	1	1%
7. Follic. Diff	1	1%
9. High B-lymph	22	22.2%
10. LPC	1	1%
11. Mantle cell	13	13.1%
12. Nodal marg. B	1	1%
13. Plasmacyt	2	2%
15. Prim. M. B-L	8	8.1%
17. Adult T-lym	4	4%
19. An-LLT	3	3%
29. Presurs TLB	2	2%
31. Subc. pann. T-lym	1	1%

Of the 99 patients there was no one EMA positive.

No CD was diagnosed before the NHL.
No CD was screened during the study.

Controls of Poland:

All controls from Poland come from occupational therapy polyclinics.
Starting date: 20th April 1999
Final date: 9th April 2001
Number of controls enrolled: 277
Males / females = 164 / 113
Mean age males 39.2 (18.5-73)
Mean age females 36.7 (18.8 – 70.4)
One control was positive for EMA and was screened during the study. We approached him 3 times, the last time just before this meeting, but until now he rejected a biopsy. So no CD was found during the study.

Discussion

J. Schmitz:

Do you have other tests for this last control person that might confirm the diagnosis of CD?

H. Szajewska:

Anti tTG is done in a small number of the participants, probably not in this control yet, but it will be done.

C. Feighery:

So Jacques do you think it worth while to do other tests?

J. Schmitz:

We were very much surprised by our low prevalence of CD in Paris. Our screening algorithm was the same as the one of C. Catassi in this Lancet publication in 1999. We started with IgG-and IgA AGA and for the positives we did the EMA tests. On a cost-effective basis it is easier to do it like that, than to do the EMA first, which is costly and time consuming.

L. Mearin:

How big is the group of the controls?

J. Schmitz:

Until now more than 3000 have been tested and planned are something like 8000-10.000. Out of the 3000 we took 560 persons and found 1 CD.

L. Mearin:

In our study, as in the MONICA study, when we are not able to have biopsies we do not consider the person as a coeliac, both in cases and in controls.

C. Feighery:

If you have an EMA positive test with no biopsy you can not say much more. On the other hand without knowing the results of total IgA in serum there is a query about the data all the time.

L. Mearin:

Is it true that in Southern Europe IgA deficiency is more common than in Northern Europe?

C. Catassi:

I never heard that.

M. Abuzakouk:

The IgA deficiency in Europe is 1: 500. But it is quite low in Japanese 1:18.000.

P. Collin:

The IgA deficiency in Finland is 1:600 and we can assume that maybe 10% of those with IgA deficiency have CD. It is 10 times more common than in the general population. This means that we must screen 5000 patients and we will miss 1 coeliac. In screening studies is not a very important issue.

**Partner's Presentation
Present position
Cases and Controls
in Areas**

Chair C. Feighery

Cases:

104 Sweden

C. Hallert, Norrköping

We studied patients in an area in Southeast Sweden. We started collecting cases in March 1999 and stopped in December 2000. During that period we collected 100 cases. We think that the response was high, over 90%. There was a predominance of male patients and they were slightly younger than the female patients were. We have to determine still the primary site and the REAL classification. Our pathologists will do that before 15th September. Two of them are here today.

We found 4 cases with a positive EMA. We identified 2 cases with CD, one before and one through screening. It may be important to define what we consider normal in terms of biopsy. One patient was on chemotherapy and has partial villous atrophy. We will repeat the biopsy within a month to see whether it is a mild CD lesion or due to a cytotoxic effect.

Discussion

L. Mellblom

I have seen the slides and it is not a normal mucosa but it is definitely not a typical coeliac lesion. So all we can do is repeat the biopsy in a period without chemotherapy and see what it shows.

C. Hallert:

The other 3 cases: would you consider them 'normal'?

L. Mellblom:

I would consider them not to be pathological.

J. Schmitz:

So 4 patients with EMA positive but zero flat mucosa.

Controls of Sweden:

In the MONICA study there were 10 controls with CD of whom 2 were known coeliacs. This has already been published.

Discussion:

L. Mearin:

I thought you had one recognised coeliac among your cases.

C. Hallert:

Yes she was known and treated for 10 years. She refused to repeat the biopsy. So we only have her vanishing antibodies and histological response after 2 years of diet. She kept a strict diet.

L. Mearin:

You have the original biopsy from years ago?

C. Hallert:

Yes.

M. Abuzakouk:

Is the histology based on 1 biopsy?

C. Hallert:

Three endoscopies.

L. Mearin:

For our study we agreed that if somebody was already properly diagnosed with CD, we would accept the person as a recognised coeliac.

J. Reehuis:

The conclusion in this case with a mild villous atrophy was no CD. In other cases in the database in the same situation the conclusion is CD.

C. Hallert:

I will get back to you about that.

Cases:

105 Finland

P. Collin, Tampere

We started in December 1998 and stopped as agreed April 14 2001.

We have 37 NHL cases. I do not know how many are non-compliant but it is about 25%.

Mean age: 60 (32-80)

I only included the lymphoma from the gastrointestinal tract.

Sites

Lymph nodes	9
Colon	2
Small intestine	1
Stomach	3
Mouth	1

REAL

2. B-cell CLL	1
4. Diffuse large B	17
5. Extranodal	1
6. Follicular centre	3
9. High-grade B	6
15. Primary B	2
17. Adult T	1
22. Intestinal T	1

The most common is the diffuse large B cell. There are still 4 REAL classifications missing but they will be ready before September.

We have one intestinal T cell lymphoma with earlier detected CD, which I will present later. From this case we have DNA. Maybe we can send it next week to Leiden.

Controls of Finland:

We have 60 controls a little bit younger than the cases.

Mean age: 50 (21-85) There was an equal number of male and female in both groups. The controls are hospital controls. They are outpatients who were not studied because of suspicion of CD or any other gastrointestinal problems. We have one middle-aged man with positive EMA and tTgA. So I am sure that he suffers from CD, but he refused a biopsy.

Cases:

206 Galway, Ireland

F. Stevens

All consecutive cases are collected from a designated area: 3 counties in the West of Ireland. I started collecting in February 2000 and I finished just before 15th April 2001.

I have collected 40 NHL cases.

18 males and 22 females.

3 of them refused to co-operate further in the study.

Age profile: females tended to be slightly older than the males.

I have not got them all classified yet, but 8 had a large B cell.

Of the serology results in NHL 33 have been done. One of them is positive.

He is a recognised coeliac who is not on a gluten free diet.

One of the NHL's has high gliadin antibodies.

Controls of Galway:

The 87 controls come from the same area. 37 males and 39 females.

Of the controls 68 have so far been studied and they are all negative. But we do have quite a large number of gliadin antibody positives in the controls and those with high levels may get a biopsy.

The age profile is as close as possible to the cases but slightly younger.

Discussion

C. Feighery:

Your controls have all been tested for EMA?

F. Stevens:

They all had IgA determinations, but the EMA has still to come through. There was an IgA deficient lymphoma, which is not included.

J. Reehuis:

You should include that case also.

F. Stevens:

But according to the protocol I could not include it if there was an IgA deficiency.

L. Mearin:

I think you are right.

P. Collin:

If we have recognised CD patients with IgA deficiency of course they are included.

L. Mearin:

I think that in one of our first meetings we made an amendment to the inclusion protocol considering IgA deficiency, but I do not know exactly what we decided.

C. Feighery:

Imagine a hypothetical situation in which a case with NHL is a coeliac proven by biopsy, but IgA deficient. I would not agree not to include the case.

H. Szajewska:

The patient could not be included anymore, as it was not send in before 15th April 2001.

J. Reehuis:

If according to a pathology report you have detected a NHL case in the study area/centre with IgA deficiency that case will enter the study.

C. Catassi:

When we wrote the protocol we thought mostly about combined primary immunodeficiency as exclusion criteria and other more severe immunodeficiencies because of the difficulties to diagnose CD in them. IgA deficiency was not considered as an exclusion criterion.

F. Stevens:

I have made a mistake then, but because we could not do EMA-tests. She does not even have a very low IgA.

C. Feighery:

Now we are even in a more complex situation.

Cases:

208 Spain

C. Farré, Barcelona

All consecutive cases from the Epilymph (Epidemiological Lymphomas) Project were recruited since May 1998 until December 1999.

The cases came from 4 Spanish hospitals in:

Barcelona	120
Madrid	42
Tortosa	8
Reus	21
Total	191cases.

The inclusion rate 97.7% in the Epilymph project. We excluded leukaemia, Hodgkin lymphomas and cases without information about gluten diet or previous CD (1.5%, 3/196) and cases with HIV+ (1%, 2/196).

<u>Cases</u>	<u>Male</u>	<u>Female</u>	<u>Total</u>
Number	103	88	191
Mean age	57.3	56.3	56.9
Age range	18-86	19-89	18-89

Primary site

Localisation: cases

Nodal 80%

Extranodal 20%

REAL classification

	<u>Total</u>	<u>%</u>
	191	100.0
Diffuse large B cell lymphoma	62	32.
Marginal zone B-cell lymphoma	19	9.9
Lymphoplasmocytoid lymphoma	17	8.9
Splenic marginal zone B-cell lymphoma	14	7.3
Follicle centre lymphoma	25	13.0
Mucosis fungoides/ Sezary syndrome	11	5.7
Anaplastic large cell lymphoma	9	4.7
Burkitt's lymphoma	4	2.1
Angiocentric lymphoma	4	2.1
Hairy cell leukemia	2	1.0
High grade lymphoma B no	2	1.0
Other	22	11.5

All cases were EMA negative.

1 case with diffuse large B cell lymphoma was diagnosed with CD at the age of 50 before the onset of the lymphoma at the age of 64 (cases 2080002).

2 cases had IgA deficiency (2080216 and 2080250).

Intestinal biopsy was not done in 2 positive tTG antibodies (89 and 30 AU) with negative EMA-tests (cases 2080007 and 2080312). One of these died and could not be studied any further.

Controls of Spain

The controls from the Epilymph study come from the same areas around the hospitals of:

Barcelona	156
Madrid	42
Tortosa	23
Reus	21
Total	242

<u>Controls</u>	<u>Male</u>	<u>Female</u>	<u>Total</u>
Number	123	119	242
Mean age	55/8	54/2	55.0
Age range	19-85	18-91	19-91

All the controls except 1 were EMA negative.

The control with EMA and DQ2 positivity was a 47-year old woman (2080046) with a heart disease and biopsy is still not done.

2 controls were for one year on a gluten free diet because of previous CD diagnosis (2080039 and 2080154).

No control had IgA deficiency.

Intestinal biopsy was not done in one control with positive tTG-antibodies (40AU) and EMA negative (2080204).

Discussion

C. Feighery:

To summarise the data, you have 2 cases and 1 control with positive tTgA but they were EMA negative.

C. Catassi:

Did you perform the tTgA determination in all the NHL and the controls?

C. Farré:

Yes in cases and in controls.

L. Mearin:

I always thought that you were working in an area, but now it seems to be 4 centres.

C. Farré:

The hospital of Barcelona represents an area. The other hospitals represent centers. So it is a mixture of area and centres.

J. Reehuis:

It is important to make a distinction in the database which cases come from an area and which are from a centre. We will have to change the UIC numbers accordingly.

J. Reehuis:

You presented today 191 cases, but I have 165 cases in the database. I have excluded the CLL's. Is that the reason of the difference?

C. Farré:

Last week we sent the definite data because we made a mistake.

Data from the database

M.L. Mearin

The data in the database dated: 03.05.01 are as follows:

CASES

Approached 1924

Studied 1334

F/M 581/749
(44% f)

Unknown 0.3%

Age F 59y (18-95)
M 59y(18-100)

<u>Primary site:</u>	<u>% (n)</u>
1. Adrenal	0.1 (1)
2. Bone	2.2 (30)
3. Bone marrow	7.4 (99)
4. Brain	1.0 (14)
5. Colon	0.8 (11)
6. Eye	0.7 (10)
7. Liver	0.7 (10)
8. Lung	1.6 (22)
9. Lymph nodes	40.4 (539)
10. Kidney	0.3 (4)
11. Spleen	3.3 (44)
12. Mouth	0.8 (11)
14. Stomach	5.6 (75)
15. Ovary	0.1 (1)
16. Testis	1.0 (14)
17. (Peri)card	0.1 (1)
18. Pleurae	0.2 (3)
19. Salivar glands	0.8 (11)
20. Skin	4.0 (54)
21. Small bowel	1.3 (18)
26. Other	16.7 (195)
Unknown/Missing	1.1 (15)/ 11.4 (152)
Total	(1334)

<u>REAL</u>	<u>% (n)</u>
1. B-prol.L	0.1 (2)
2. B-CLL/SLL	4.6 (62)
3. Burkitt	0.9 (12)
4. Diffuse B	34.9 (465)
5. Extranodal	3.1 (42)
6. Follicular centr.	14.2 (189)
7. Follic.diff	1.3 (17)
8. Hairy cell	0.1 (2)
9. High B-L	3.7 (49)
10. LPC	4.0 (53)
11. Mantle cell	4.5 (60)
12. Nodal marg. B	1.9 (26)
13. plasmacyt.	0.3 (4)
14. precurs.B-LL	0.1 (1)
15. Prim. M. B-L	1.1 (15)
16. Splenic	1.3 (17)
17. Adult-T-L	0.4 (6)
18. An-LLO	0.9 (12)
19. An-LLT	0.8 (11)
20. Ang im-T	0.7 (10)
21. Hepatosplen	0.1 (1)
22. int.T, ent-	0.1 (2)
23. Int. T, ent+	0.2 (3)

<u>REAL</u>	<u>% (n)</u>
25. LGLL,NK	0.1 (1)
26. Mycosis fung	1.5 (20)
27.NK/TL	0.1 (1)
28. Perf. T-L	1.3 (17)
29. Prec TLB	0.4 (5)
30. Sezary syndr.	0.1 (1)
31. Subc. Pann. T	0.1 (1)
33. Angioc. Lymph	0.4 (6)
34. B-cell, NOS	1.1 (15)
35. T-cell, NOS	0.2 (3)
Unknown/Missing	3.0 (40)/ 12.2 (163)
Total	(1334)

As you will see from the following table we still miss the EMA results of 209 cases (16%): We should improve those results.

<u>IgA deficiency</u>	<u>%</u>
Yes	0.4
No	31
Missing	68
<u>High IgA-AGA</u>	<u>%</u>
Yes	2
No	48
Missing	50
<u>High IgG-AGA</u>	<u>%</u>
Yes	0.4
No	22
Missing	78
<u>High IgA-EmA</u>	<u>%</u>
Yes	0.6
No	84
Missing	16 (209)!
<u>High anti-tTG</u>	<u>%</u>
Yes	0.6
No	28
Missing	71

In the 1334 Cases recruited until 3rd May 2001:

CD:

Yes	17
No	83%
Missing	212 (16%)(!!)

CD recognised prior to study: 12, with the following UCI

1011307	1040103
1013009	1060002
1013012	1060016
1013026	1110036
1029001	2060024
1030045	2080002

CD found by screening through the study: 5

1010047
1011114
1013008
1030011
1060065

This table shows the data of the 17 cases with NHL and CD

UIC	S	Age	Prior	Gfd	Site	REAL	EmA	Date Sbb	HLA DQ
1010047	F	56	-	-	Small Bowel	EATL	+	09.99 Sva	2
1011114	F	55	-	-	Small Bowel	EATL	+	?99 Sva	2
1011307	F	70	+	+ 7y	Liver	Diffuse B	-	01.93 Sva	2
1013008	F	68	-	-	Bone Marrow	Diffuse B	+	?99 Sva	2 8
1013009 U.J.(+)	F	47	+	+	Small Bowel	EATL	nd	? Sva	2
1013012 refr.cd (+)	M	62	+	+ 2y	Small Bowel	EATL	nd	1997 ?	2
1013026 U.J. (+)	F	49	+	+ y?	Small Bowel	EATL	nd	?	2
1029001 D.H.	M	50	+	+ 8y	Lymph Nodes	Diffuse B	-	10.87 Pva	? o.w.
1030011 D.H.	F	63	-	-	Lymph Nodes	Diffuse B	+	11.99 Sva	2
1030045	M	59	+	+ 2y	Skin	Mycos. Fung.	nd	08.99 Sva	2
1040103	F	75	+	?	?	?	-	? ?	?
1060002	F	67	+	+ 3y	Omentum	Diffuse B	nd	06.95 Pva	?
1060016 D.H.	F	65	+	+ 22y	Lymph Nodes	Diffuse B	-	1977 Pva	? o.w.
1060065 (+)	M	64	-	-	Skin	Angio. Lymph	+	05.00 Sva	?
1110036	M	38	+	?	Lymph Nodes	Fol. Centr.	nd tTG-	?	?
2060024	F	48	+	+/-	Small Bowel	EATL	+	08.91 Sva	2
2080002	F	65	+	+ 15y	Lymph Nodes	Diffuse B	-	?	2

CD prior to the study	12	CD through the study	5
GFD before NHL	9/10		0/5
EATL (REAL 23)	4	EATL (REAL 23)	2
D.H. 3/6	3		
CD in the family 2/9	1		1
Ulcerative jejunitis 2/11	2		

None of the 5 unrecognised CD cases were following a GFD. That is logical. From those 12 cases of recognised CD we only have information about the GFD in 10 cases. It is important to get additional information from the 2 cases.

Discussion

C. Catassi:

Are those 3 cases with refractory sprue included?

L. Mearin:

Yes.

J. Schweizer:

From what country does case 1110036 come from?

L. Mearin:

Yugoslavia.

Chr. Mulder:

We should get data about the mean duration of the GFD.

M. Abuzakouk:

What about the information in the 9 cases?

L. Mearin:

The information is quite rough. Just if the patient was following a GFD yes or no and in some cases the physician might have said that the diet was very poor or badly followed. From the 10 cases with information about the diet, 9 were following a GFD.

J. Schmitz:

It should be possible to go back to the patient and the doctor to learn more about the diet. It is of crucial importance.

L. Mearin:

We should get the information about the diet in the last 6-12 months before the diagnosis of NHL.

P. Collin:

We agreed to use the 'Holmes' protocol to assess the diet.

F. Stevens:

Could we assess the diet 1 year, 6 months and 3 months before the diagnosis of NHL?

Chr. Mulder:

I do not think that that makes sense. The duration of the development of the lymphoma is not 1 year.

F. Stevens:

Recognised coeliac patients on a poor diet or no diet go back on a diet when they begin to get symptoms (from the lymphoma), which maybe 6 months before the diagnosis.

L. Mearin:

We will get a copy of the 'Holmes' protocol that was send to every partner who has cases with CD and NHL and we will discuss it later.

J. Schmitz:

Is there a chance that the people who have had a lymphoma had an intestinal biopsy at the time of the diagnosis, so that we can know the state of the mucosa?

L. Mearin:

For a couple maybe. J. Schweizer has some cases.

J. Schweizer:

The problem with my cases is that they were refractory coeliacs probably on a strict diet.

L. Mearin:

If you look at the kind of lymphomas, 4 of the 12 recognised coeliacs had an EATL and 2 of the 5 unrecognised had an EATL as well.

Chr. Mulder:

What is our definition of an EATL?

N. Brousse:

By microscopic revision. Refractory sprue itself is not recognised as a lymphoma at the moment.

Chr. Mulder:

Some of our refractory CD patients develop extra-intestinal lymphomas, but with the same immunohistology as the EATLs. We consider them as EATLs, but the actual definition of EATL is a small bowel lymphoma.

N. Brousse:

We should accept the international classification of NHL at the moment: an EATL is an enteropathy associated T cell lymphoma.

Chr. Mulder:

For this study we should, but maybe for future studies we should do immunohistology in the extraintestinal lymphomas to see whether the T cells are intestinal or not.

L. Mearin:

That is a very important point. Should we send material of our 17 cases with NHL and CD to Nicole Brousse to study this?

Chr. Mulder:

We definitively should.

L. Mearin:

The group should make a decision on this point.

P. Collin:

Could you specify what you mean by "send material"?

L. Mearin:

Slides of the lymphoma.

F. Stevens:

Just from the cases with CD and T cell lymphoma?

L. Mearin:

No, from all the 17 cases with CD and a lymphoma of any kind.

N. Brousse:

I have 8 of them already.

J. Schmitz:

Coming back to the figures. Here are 6 EATLs but in your former sheet there were 3 EATLs (0.2%) of the 1334 lymphomas screened.

J. Reehuis:

The 3 cases of Joachim which are counted in the 17 positive cases died and were not screened: they are not included in the 1334 screened cases, but they have been counted as CD patients with lymphoma (EATL), so 6 EATLs in total.

L. Mearin:

It is interesting that 3 of the 12 recognised coeliacs before the study that developed lymphoma had dermatitis herpetiformis (DH). What is the frequency of DH in CD?

P. Collin:

It varies in different series and different countries: in Sweden and England it is less frequent, than in Finland where the frequency is 25%. I do not know what the reason is. Maybe it depends on the skills of the dermatologists, or of the gastroenterologists.

P. Ciclitira:

In the UK it is about 5%.

L. Mearin:

2 of the 17 cases have family members with CD and 2 have ulcerative jejunitis.

J. Schmitz:

It is interesting, because familiarity has been considered as a risk factor for malignancy in CD.

L. Mearin

We still miss information about familial CD in 9 cases. We will have to get this information.

The next 2 tables show the primary site and REAL classification of the 17 cases with NHL and CD.

<u>Primary site</u>	<u>n</u>
3. Bone marrow	1
7. Liver	1
9. Lymph nodes	5
20. Skin	2
21. Small bowel	6
26. Omentum	1
Total	17

<u>REAL</u>	<u>n</u>
4. Diffuse B	7
6. Follicular centr.	1
23. EATL	6
26. Mycosis fung.	1
33. Angioc. Lymph	1
Missing	1
Total	17

L. Mearin

Case 1013012 is a refractory CD. CD was established in 1997.

He was following a GFD at the time of the lymphoma.

Chr. Mulder:

The EMA test of this patient was checked 2 years ago and was negative then. I will look for this data.

L. Mearin

One case with DH comes from Italy, another from Derby and another one from Dublin.

We still have very little information about the case from Sweden but I am sure we will get it.

The next table shows an overview of the cases and controls per partner/country: 1334 cases and 9597 controls.

	NHL	CD (%)	Controls		CD (%)	
Neth 101	218	7 (3.2) p4 (1.8)	1440	1000	?	2(0.2)p0
Ital 102	353	1(0.3) p1(0.3)	3282		18-17?(0.5) p2(0.1)	
De103 Lo203	101 24	2(2.0) p1(1.0) 0	457 0		3(0.7) p2(0.4) 0	
Sw 104	98	1(1.0) p1(1.0)	1894		10 (0.5) p2(0.1)	
Fin105	37	1(2.7) p1 (2.7)	60		0	
DubBe 106 Gal 206	85 37	3 (3.5) p2(2.4) 1 (2.7) p1	1823 75		11(0.6) p2(0.1) 0	
Barc 208	186	1(0.5) p1	267		2(0.7) p2	
Fran 109	73	0	402		1(0.3) p1(0.3)	
Pol 110	86	0	277		0	
Yug 111	36	1 (2.8) p1(2.8)	60		1(1.7) p1(1.7)	
T	1334	17(1.3) p12(0.9)	9597		47(0.5) p12(0.1)	

The 2 different numbers for the controls in the Netherlands indicate both the 1000 blood donors studied and published by Chris Mulder (2 with CD) and the other group of 1440 from the general Dutch population Joachim Schweizer told you about earlier.

An overview of the primary sites and the REAL classification.

Primary site	1334 NHL %	17 NHL and CD %
Lymph nodes	40.4	5.8
Bone marrow	7.4	5.8
Stomach	5.6	0.0
Skin	4.0	11.7
Spleen	3.3	0.0
Liver	0.7	5.8
Small bowel	1.3	35.2
Omentum	0.0	5.8

REAL	1334 NHL %	16 NHL and CD %
Diffuse B	34.9	43.7
Follicular centr.	14.2	0.6
B-CLL/SLL	4.6	0.0
Mantle cell	4.5	0.0
EALT	0.2	35.2
Angioc. Lymph	0.4	0.6

Partner	NHL	CD (%)	Prior CD (%)	Controls	CD (%)	Prior CD (%)
101	218	3.2	1.8	1000	0.2	0
102	353	0.3	0.3	3282	0.5	0.1
103	101	2.0	1.0	457	0.7	0.4
203	24	0	0	0	0	0
104	98	1.0	1.0	1894	0.5	0.1
105	37	2.7	2.0	60	0	0
106	85	3.5	2.4	1823	0.6	0.1
206	37	2.7	2.7	75	0	0
208	186	0.5	0.5	267	0.7	0.7
109	73	0	0	402	0.3	0.3
110	86	0	0	277	0	0
111	36	2.8	2.8	60	1.7	1.7
T	1334	1.3	0.9	9597	0.5	0.1

Centres	NHL	(CD)%	Controls	(CD)%
101	83	(5)7.2	1000	(2)0.2
102	353	(1)0.3	3282	(17)0.5
103	101	(2)2.0	457	(3) 0.7
203	24	0	0	0
105	37	(1)2.7	60	0
106	85	(3)3.5	1823	(1)0.6
206	37	(1)2.7	75	0
110	86	0	277	0
111	36	(1)2.8	60	(1)1.7
T	842	(14)1.7	7034	(24)0.3

Discussion

Chr. Mulder:

Interesting is the follow-up of the blood donors: a third one developed a March II intestinal lesion and another one with very high IgA antigliadin antibodies developed 3 years after the study an intraepithelial lymphocytosis and a partial villous atrophy. It makes the discussion a bit more difficult because it gives an impression that if you wait long enough you have more CD among the controls.

L. Mearin:

What we are reporting here is an absolute minimum of CD in the populations that we study.

Chr. Mulder:

I would like to know how many Tcell lymphomas of the stomach have been recognised in the whole study. Some of our refractory coeliacs have developed a NHL in the stomach.

L. Mearin:

We did not find any NHL in the stomach among the cases with CD, but we can have a look at that.

R. Brand:

I would like to explain my letter to the partners with the deadline for the data I just gave to everybody because it rather fits in now.

Normally all the individual cases and controls go into the database. But in this study there are also the bulk or tabulated controls. They will not be put in the database. This means that there are 2 flows. One are the individual cases and controls. The other are the tables of the controls with gender and age categories. Those go to me. I put everything together in a statistical data centre: the database from Janneke Reehuis and the bulk of tabulated controls. I have printed from the statistical database the numbers that I think would enter the analysis per partner. This comes in two parts. One part is without information about EMA+ and CD+, because the deadline for this information is in September. However, the other part containing information on being a case or control, with gender and age should be complete at this very moment. I need confirmation from you to make sure that what I have in my statistical data centre is correct.

For that reason I want a letter signed by all the partners stating that you fully agree on every single number on it. You may send the signed letter to office in Leiden.

L. Mearin:

What Ronald said is very important, because it is the quality control of our data.

Presentation of all cases with NHL and CD

Chair: J. Schmitz

All 17 cases will be discussed.

The Netherlands

J. Schweizer:

I like to start with the cases that were recognised as a coeliacs before they developed a lymphoma and entered the study.

UIC 1013009 from Arnhem

Female, born in December 1952. She was 47 years when diagnosed with NHL. CD was diagnosed 15 years before our study. She was a refractory coeliac patient, not responding to the diet. She was diagnosed with an EATL and died before she could be interviewed for the study. Her DQ is compatible with CD. Material of her has not been sent to Paris yet.

Discussion

C. Feighery:

Was she unequivocally a coeliac patient? Was she ever EMA positive?

Chr. Mulder:

Yes, 15 years ago. Her small bowel histology proved that she was a coeliac. When we saw her the first time she was losing weight, but as a matter of fact

she was overweighted. Her body mass index was 25 when we saw her with partial villous atrophy.

L. Mearin:

How did you define this EATL?

Chr. Mulder:

According to the current definition.

N. Brousse:

I think that this case has not been reviewed.

J. Schweizer:

I'll inform you about the cases and their numbers, which have been sent.

UIC1013026 from Arnhem

Female, born July 1950. She was 49.5 years old when she was diagnosed with the lymphoma. She was diagnosed as a coeliac 5-6 years before. She was on GFD. I talked to her dietician, but it was difficult to know how well she kept the diet.

Chr. Mulder:

Both these patients saw a dietician outside the hospital who is a coeliac herself and very committed to the GFD. She is controlling and checking the diet in over 200 coeliacs in our department. This dietician trusted the two patients as to keeping the diet.

J. Schweizer:

She was on a diet for 5 years. Her HLA-DQ is compatible with CD.

C. Catassi:

Were both patients EMA negative at the time of the lymphoma?

Chr. Mulder:

Yes.

L. Mearin:

Was the EMA negative or not done?

Chr. Mulder:

It was not done during the period of the study, but it was done a month before and it was negative.

N. Brousse:

Did she ever improve her symptoms on a GFD?

Chr. Mulder:

Yes no doubt, but 3-4 years later she deteriorated and she developed refractory CD.

J. Schmitz:

Someone, for example our European co-ordinator, should have the complete medical history of every case with NHL and CD. A document of 2-3 pages as it is very important to document our findings.

L. Mearin:

It is important what Jacques is telling us. For these very special 17 cases we should have as much information as possible. Write the history of the patient and send it to Leiden.

N. Brousse:

And all the histology reports.

Chr. Mulder:

If you want to have the earlier small bowel biopsies we can send them to you.

P. Collin:

We must remember that it is difficult to draw conclusions about the connection between the GFD and lymphoma. The starting-point of this study was totally different. We will have in our 17 patients an incomplete retrospective diet history.

J. Schmitz:

But the comment that Chris Mulder made about the assessment of the GFD by a dietician is reliably and important.

UIC1013012 from Arnhem

Male, born in July 1937. 62.5 years old when diagnosed with an EATL. He was diagnosed 3 years before with CD. He is currently on a strict GFD. He relapsed while on GFD.

Chr. Mulder:

He had almost normalised biopsies 2 years before. I have no doubts about his dietary compliance. We saw him because he did not do very well and at endoscopy he had many ulcerations in the oesophagus, stomach and in duodenum. We took many biopsies from his stomach and jejunum, but interestingly only 1 biopsy from the stomach was positive for T cell lymphoma. I was quite certain about the diagnosis of NHL in his case. We put him on therapy, however the haematologists were not convinced it was a NHL and they asked for new biopsies 2 weeks later. At that moment there were no ulcers in his stomach and all the biopsies of the stomach were negative for T cell lymphoma. However, the lymphocytes in his stomach, oesophagus and duodenum were CD3, and after long debates the haematologist accepted the diagnosis and started chemotherapy. After the first course of CHOP he perforated his jejunum and had to be operated. All samples taken out of his jejunum were positive for NHL. By giving him treatment we proved unfortunately that he had an EATL.

Discussion

P. Ciclitira:

Should one consider treating these people with steroids before giving them chemotherapy?

Chr. Mulder:

If you look at the history of the EATLs the survival is extremely poor and a number of them perforate after one course of chemotherapy. I think the whole approach to those patients must change to improve their survival rates. I think it is quite dangerous to treat these patients with CHOP.

J. Schmitz:

Those 3 EATLs were under a strict GFD for many years?

Chr. Mulder:

The first one for 15 years, the second one for about 5 years and the third one for about 3 years. We are now looking at their biopsies to look retrospectively for the CD3+ cells.

UIC1011307 region of the cancer centre.

Female, born in December 1929. She was diagnosed 7 years before with CD because of a long medical history including well-known coeliac complications as abortions, aphtae, diarrhoea, iron deficiency etc. She was put on a GFD. She was not advised by a dietician, but used a well-known list in our country for gluten free products. She was very strict with it. So I do not question her diet. She also said that if she would use small quantities of gluten she got complaints. She was diagnosed with a diffuse large B cell lymphoma nodal. It was not in the intestine. I screened her for antibodies, which were negative. She was DQ2+.

Chr. Mulder:

What was the site of the lymphoma?

J. Schweizer:

In the liver and she had metastases in the lymph nodes. It was on both sides of the diaphragm, but without bone marrow infiltration.

N. Brousse:

A diffuse large B cell lymphoma is very rare in the liver. Was it a large tumour?

J. Schweizer:

I do not recall. I would have to read the pathology report again. They took a percutaneous biopsy so it must be some centimetres.

N. Brousse:

Was the intestinal biopsy performed at the same time?

J. Schweizer:

It was performed 7 years before and was not repeated. I had no reason for the study to do it, as she was negative for antibodies.

C. Catassi:

A general remark: it would make sense to say 'presenting' site rather than 'primary' site.

The cases found through the study.

UIC1013008

Female born in September 1931 with a history of aphtae, joint complaints and lichen ruber. She developed symptoms of anorexia, night sweats, lassitude and weight loss and was diagnosed with a diffuse large B cell lymphoma. When she was enrolled in the study her EMA test was positive as for tTGA and she had a subtotal villous atrophy on jejunal biopsy. She is currently on a GFD diet and has a good response. The primary site was in the bone marrow.

Discussion

N. Brousse:

So we have a second case where the primary site is unusual. NHL in the liver or in the bone marrow are not frequent.

J. Schmitz:

I noticed that she had anorexia. How did it improve?

J. Schweizer:

I do not know.

E. Fabiani:

You said that she had an autoimmune disease?

J. Schweizer:

On the skin, lichen ruber.

UIC1011114

Female, born in February 1944. She was not known to have CD. She was healthy before and presented with an intestinal bleeding. She was eventually operated upon. A jejunal resection was done and she was diagnosed with an EATL. A few weeks later her EMA test was positive and tTGA were positive. She was HLA DQ2. The primary site (the presenting site) of the lymphoma is the small bowel.

Discussion

F. Stevens:

Was the tumour detected on an X-ray? Why did they decide to operate?

J. Schweizer:

She was circulatory instable when she came into the hospital. They did intravenous reanimation and upper and lower endoscopy and an angiography as she kept on bleeding. After a large number of packed cells they decided they had to operate.

E. Fabiani:

Was she a diabetic?

J. Schweizer:

Yes

E. Fabiani:

So you know the time of the diagnosis of diabetes?

J. Schweizer:

I do not.

Chr. Mulder:

How was she treated?

J. Schweizer:

CHOP, but I did not follow her after the study.

J. Schmitz:

So this would be an EATL who did not die.

Was she operated in 1999?

J. Schweizer:

I expect that she is deceased but I am not sure. I can ask.

UIC1010047

Female, born in November 1943. She was 56 years old when she was diagnosed with a lymphoma. She was not known to have CD, but had a history of anorexia, chronic diarrhoea, distended abdomen and at least one clinical depression. She was referred to a general hospital and was investigated for CD by duodenal biopsy, which was compatible with CD, so

she was treated with a GFD. One month later she became seriously ill, was sent to our centre and diagnosed with an EATL. She was EMA+, tTgA- and DQ2+.

Chr. Mulder:

We agreed before that to be considered as a case of recognised CD before the study the interval between the CD diagnosis and the lymphoma should be 6 months or longer.

J. Schweizer:

The interval in this case was 1 month.

Italy

E. Fabiani

UIC1029001

A Caucasian male with nodular lymphoma diagnosed with associated CD before entering the study.

Patient's clinical history (as child and adolescent)

- Chronic diarrhoea during the first 2 years of life
- Recurrent abdominal pain up to 10 years of age
- Iron deficiency
- Some mood problems (depression and irritability)

Patient's past clinical history (as adult)

- Always complaining of an early feeling of gastric filling
- 1987 (first six-month period): diagnosis of DH (the skin lesions started 2 years before)
- October 5, 1987
Upper GI endoscopy: diagnosis of CD (age 41)

Histology report

(biopsy taken from the 2nd part of the duodenum)

PVA with crypts elongation

Slight decrease of PVA of the cells height

Increased IELs count

A diagnosis of gluten enteropathy was made.

* No information is available about neither the CD serology marker that was performed nor its results (it could have been the AGA test)

* A GFD was started and there was a significant clinical improvement (weight gain, DH recovering, no more mood problems)

* Compliance to GFD: always strict

I have good reason to believe him because:

* NHL well controlled

* CD & DH: no problems at all,

* Checked CD serology (AGA, EMA & anti-tTG): negative

* Stored a whole blood sample for HLA typing

Patient's data on NHL

- Date of diagnosis: January 1999 (age: 49)
- Name of laboratory: Dept of Oncology, Modena
- History leading to NHL: enlarged left neck lymph nodes
- Time from symptoms to diagnosis: 1 year
- NHL primary site: lymph nodes
- Other sites: abdominal lymph nodes & muscles
- Histology (REAL): diffuse large B-cell
- Staging (Ann Arbor classification): III A
- Associated disease: none
- Serological screening (AGA & AEA): negative (GFD)

He did not undergo a tTG test.
From this patient I send blood to Leiden for HLA typing and we are waiting for the result.
J. Schweizer:
We had problems picking up the DNA.

United Kingdom

Derby (L. Mearin)

1030011

Female, born in June 1936. Not known to have CD before the study. She presented with a diffuse B lymphoma of the lymph nodes in November 1999. EMA+. History: signs of anorexia, chronic diarrhoea and chronic lassitude. The small bowel biopsy in November 1999 showed subtotal villous atrophy. HLA DQ2. She has DH.

N. Brousse:
I have the biopsy of CD diagnosis, which was typical and I have the biopsies of the lymphoma.

1030045

Male, born in March 1941. CD was established in August 1996 with good response to the GFD. Two years later skin problems, which turned out to be mycosis fungoides, a lymphoma of the skin. The EMA was not done, as he was known to have CD.

Discussion

N. Brousse:
This is a very interesting case. I have slides from the skin but not from the small bowel.
L. Mearin:
There was a small bowel biopsy in 1999, so there must be more material.
J. Reehuis:
Should there not be EMA results?
L. Mearin:
That is not necessary if it is a case with CD recognised prior to the study.

Sweden

C. Hallert

1040103.

A female born in July 1925. She was diagnosed with CD about 10 years ago. She got a lymphoma one year ago. She was on a strict GFD as far as I know. I will have the information at the next meeting.

L. Mearin:
The information should be in by September 15.
N. Brousse:
Do you know the site of the lymphoma?
C. Hallert:
I will have it later.

Ireland

M. Abuzakouk, Dublin

UIC1060002

Female, Caucasian, born in September 1931. Diagnosed with CD by biopsy in 1995 with partial villous atrophy. She was put on a strict GFD shortly after the diagnosis.

In December 1998 she developed a B cell lymphoma in the omentum. She was treated with surgery and had 4 courses of chemotherapy. A few months after her remission she had a recurrence and again she was treated with chemotherapy. But she continued to deteriorate and died.

1060016

Female, Caucasian, born in August 1934. She was diagnosed with DH in 1977. She was put on a strict GFD with good results. In July 1999 she developed a large ulcer at the left tonsil. That was biopsied and it was proven to be a B cell non-Hodgkin lymphoma. She received chemotherapy and radiotherapy with good response. She had a recurrence in the stomach some months after her remission. Again she received chemotherapy. I saw her recently and she is quite well.

C. Feighery:

Do you have good evidence that her diet was strict?

M. Abuzakouk:

This lady was involved in another study looking at bone mineral density in DH and we actually evaluated her GFD twice. So I have good evidence that her diet was strict.

J. Schmitz:

We are partly answering the question about a strict or not strict diet, because here we have a diet that was very strict.

L. Mearin:

Ideally, we would like to be able to tell the number of gluten in grams per day that were consumed. But that is impossible to assess.

UIC1060065

A male Caucasian born in January 1936. He was diagnosed with dermatomyositis in 1999. He was treated with steroids and antimalarials with good response. That treatment was stopped 9 months later when he went into remission. In April 2000 he became unwell. He had constant diarrhoea and findings of endoscopy were suggestive of inflammatory bowel disease. Four weeks later AGA and EMA were found to be strongly positive. The small bowel biopsy showed marked villous blunting and focal increased epithelial lymphocytes. He was placed on a GFD. However he continued to deteriorate and lose weight. He developed a nodular skin lesion on his left arm. The biopsy showed that it was an infiltrated angiotropic T cell lymphoma. The teams involved decided not to give any chemotherapy, but just to give supportive measures. He continued to deteriorate and died 3 months after the diagnosis of CD.

Discussion

J. Schmitz:

Was there no repeated intestinal biopsy? The histology of the first biopsy is not very conclusive.

M. Abuzakouk:

We have only one.

J. Schmitz:

It is blunting. In this kind of patient, which is becoming worse you expect some very flat mucosa and it is not the case.

Chr. Mulder:

In our patients developing lymphoma with refractory CD the majority had partial villous atrophy while they were developing an EATL. Only one had total villous atrophy.

P. Collin:

Did you search for a malignancy when you discovered the dermatomyositis?

M. Abuzakouk:

We were not very much involved. All I could get was the information from the records.

N. Brousse:

I have the slide here. There is no infiltration by CD3+ lymphocytes. Very few of them. It is not a well-orientated biopsy.

J. Schmitz:

Would you say that this is against the diagnosis of CD or that this is typical for an EATL or an other lymphoma?

N. Brousse:

I do not find it compatible with CD because there is no hypercellularity in the lamina propria, there is no crypts hypertrophy and it is not atrophy.

L. Mearin:

Could this be a Marsh type IV going to V?

Chr. Mulder:

You can not conclude anything with such slides.

P. Collin:

When has an increase of intraepithelial lymphocytes been a diagnostic criterion for CD? Not according to the ESPHGAN. I do not think that the literature is very convincing that every coeliac patient always has an increase in intraepithelial lymphocytes. I do not think that it is convincing. At the moment the diagnostic criteria are: villous atrophy and crypts hyperplasia.

N. Brousse:

I have seen a lot of CD. But here the epithelium is normal. There are normal villi. There is no increase in plasma cells.

P. Collin:

What would you say when you have such a lesion with EMA+?

L. Mearin:

CD.

Chr. Mulder:

No, I would say this is not a good orientated biopsy.

C. Feighery:

There are certain atypical features for an ordinary CD, but we have to recognise the possibility that CD may be patchy. The real definition of CD is an intestinal lesion that improves clinically and histologically after a GFD. Obviously we did not have the opportunity in this patient to check that. I do not know what you can conclude, but I do not think you can say this is no CD here. I agree with Pekka

L. Mearin:

An important point is that he is one of our 17 and we have to decide if we have 17 or 16.

J. Schmitz:

The main problem here is the meaning of the EMA positivity.

Because of EMA+ we have to take it seriously, otherwise we will not be convincing at all.

N. Brousse:

Do not forget he has an immune disease: dermatomyositis.

L. Mearin:

He was treated with corticosteroids.

P. Ciclitira:

Was the patient on steroids at the time the biopsy was taken?

M. Abuzakouk:

He was on steroids in January 1999. He had his biopsy in May 2000.

Ireland

F. Stevens, Galway

UIC2060024

A Caucasian female born in September 1951. She has a son who has CD. She had several children, one with spine bifida. She presented in 1991 with pyrosis and nausea for a year. She was overweight and anaemic. In August she had an endoscopy and subtotal villous atrophy and was Helicobacter Pylori positive. She was advised to follow a GFD. She did not believe that she had CD and kept asking me if I was sure of the diagnosis. She thought she did not need to be on a diet. The last time I saw her prior to the diagnosis of her lymphoma was in 1995. She claimed to be well. She claimed to be on a sort of GFD, but the antigliadin antibodies were terrible high. Sometime around February 2000 she went to someone of the coeliac society because she was not feeling well and perhaps she should start a GFD. This was a few months before she presented in June with weight loss. But even then did she deny being a real coeliac. She had an ultrasound and a barium follow through, which showed a proximal jejunal obstruction. At that stage her blood was EMA+ borderline positive for AGA. She had a laparotomy in

June and she had a circumferential white tumour regular in surface. It was pin tracking through the wall. She had fleshy lymph nodes and extensive mucosal ulceration.

N. Brousse:

It is a clear-cut case of CD because of the flat mucosa. I could not perform the phenotype of the lymphocytes because I had no special slides for immunohistochemistry.

F. Stevens:

The resection margin showed subtotal to total villous atrophy. I have been seeing her in the clinic since she has recovered from CHOP. She put on a lot of weight again. She then began to complain two months ago. I did an endoscopy within the last 6 weeks and her villi are present now and her EMA and antigliadin antibodies are negative, although she still has doubts about being a coeliac and needing a GFD.

Spain

C. Farré, Barcelona

UIC2080002

Female, born in April 1934. She was recognised to have CD at the age of 50. She has been on a GFD for 15 years. The serological results were negative. She was DQ2+. At the age of 64 she was diagnosed with a large B cell lymphoma. The patient has died. At the moment I do not have more clinical information. The information about the CD is only through the interview and through confirmation by the family. We do not have the initial biopsy and it will be impossible to get it, because I do not know where it was done.

N. Brousse:

It is a large B cell lymphoma, which is the most frequent type of lymphomas.

Finland

P. Collin

1040035

Male, born in April 1939. In the pre-coeliac era in 1987 he was suffering from atrial fibrillation and heartburn. This is the only time I saw the patient. He went for an endoscopy and a duodenal biopsy. I took a routine duodenal biopsy showing some mucosal atrophy but not really diagnostic for CD: here you can see the 14-year old slide.

N. Brousse:

You can see plasma cells in the lamina propria and increased intraepithelial lymphocytes.

P. Collin:

The pathologist wanted a second biopsy with a capsule for diagnosis, but the patients was reluctant.

Three years later he also suffered from indigestion and a new biopsy was done in March 1990 showing subtotal villous atrophy. The third biopsy was taken in July 1990. There was no recovery at all. But dietetic investigations showed that he was only on a semistrict diet. He was advised to adhere to a strict diet. He did not come back for a check up.

In 1999 due to melena a surgeon carried out endoscopy, but did not take biopsies from the small bowel. In 2000 the colonoscopy was normal and also the small bowel enema.

In the beginning of 2001 he had a weight loss of 15 KG and an abdominal mass was discovered. He was operated in February 2001. Antibodies against transglutaminase and endomysium were negative. It seems that the pathologist said that outside the tumour there seemed to be normal mucosa, but this was difficult to assess because of the local necrosis. So I am quite sure that his NHL is a complication of CD: CD3+ CD4++ CD68. He is not doing very well. He needs enteral nutrition and chemotherapy and I think that the prognosis will be very bad.

Discussion

C. Feighery:

Was the lymphoma involving the intestine?

P. Collin:

Yes, the small bowel.

N. Brousse:

Was there any biopsy of the colonoscopy in 2000?

P. Collin:

I do not remember but there might be some. I will check it.

L. Mearin:

This case of P. Collin was not included in the 17 cases that I showed, because there were difficulties to get the dietetic history.

J. Schmitz:

So perhaps we have 18 cases.

C. Hallert:

Ewa Grodzinsky, who is not here today, conducted a study following coeliacs for 10 years and found that you should rely on the patients' statement as to the GFD.

Chr. Mulder:

It depends on how you question. If you ask the patients: "are you on a GFD?" they will always confirm that. But if you ask: "is it a difficult diet? how many times do you make mistakes?" Then the answers might be more reliable. The answers can be predicted by the way of questioning.

J. Schmitz:

Before we stop I would like to say that the presentations were absolutely fascinating. Few of those who presented these cases alluded to the story during childhood. I think it will be very important to get to know as much as possible about what happened during childhood.

Saturday 30th June 2001

Chair: C. Farré

Quality control study

Mohamed Abuzakouk

I would like to thank everybody in taking part in this small project.

We sent 9 sera samples to the 12 laboratories involved and to an external laboratory. The samples were from 8 different individuals.

The samples were	Code
Strong EMA+	1
Weak EMA+	2
EMA- due to GFD, previously EMA+	3
ENA (extractable nuclear antigen) +	4
ANA (antinuclear antibody positive):	5
Mit (mitochondrial antibody) +	6
SM (smooth muscle antibody) +	7
IgA deficient	8
Normal: EMA negative from a healthy person	9

We asked each lab to perform EMA and tTG according to their local protocol. There was a good agreement between laboratories as to the EMA test (see table below). All the labs reported the strong EMA+ sample as such. However lab F and the external lab reported the weak EMA+ sample as negative. Lab B reported the smooth muscle antibody positive sample as EMA+.

IgA EMA

		Strong EMA+	Weak EMA+	pEMA+, nEMA-	ENA+	ANA+	Mit+	SM+	IgA-	normal
	Centre/ Sample Codes	1	2	3	4	5	6	7	8	9
1	A	yes	Yes	no	no	no	no	no	no	no
2	B	yes	Yes	no	no	no	no	yes	no	no
3	C	yes	Yes	no	no	no	no	no	no	no
4	D	yes	Yes	no	no	no	no	no	not done	no
5	E	yes	Yes	no	no	ANA+	no	SM+	no	no
6	F	yes	No	no	no	no	no	no	no	no
7	G	yes	Yes	no	no	no	no	no	no	no
8	H	yes	Yes	no	no	no	no	?SM+	no	no
9	I	yes	Yes	no	ANA+	no	no	SM+	no	no
10	J	yes(80)	trace(2.5)	no	no	no	no	no	no	no
11	K									
	a(neg<10)	1280	10	ANA+	ANA+	ANA+	SMA+	SMA	no	no
	b(neg,20)	320	80	no	no	no	no	no	no	no
12	L									

Discussion

L. Mearin:

The weak positive was from a coeliac patient consuming gluten?

M. Abuzakouk:

Yes. Recently diagnosed and put on a GFD. All the serum samples were obtained from coeliac patients, who were biopsy proven coeliacs.

L. Mearin:

Did you do titrations?

M. Abuzakouk

We do not do titrations.

L. Mearin:

You have the sera. Perhaps in the more disputable cases, like NR 2, you could do titrations.

C. Feighery:

Immuno florescence is a subjective test. To decide if something that is weak positive is in fact negative is difficult. What we should do is to rely on the tTGA data, which depends on an ELISA test, and the subjectivity is not present.

P. Ciclitira:

It is difficult to evaluate where the cut-offs on the negative points should be. A year ago the American Gastroenterology Association showed that in America if you send samples to commercial laboratories the sensitivity of EMA is only 50%.

IgA AGA

		Strong EMA	Weak EMA	pEMA+ nEMA-	ENA+	ANA+	Mit+	SM+	IgA-	normal	positive
	Centre/Sample Code	1	2	3	4	5	6	7	8	9	
1	A	>100	23.9	1.9	3.9	2	0.8	9.5	0.1	1.2	>5
2	B	22	1	1	1	1	2	3	1	1	>7
3	C	1052	36.8	15.4	10.4	7.9	2.9	69	0.2	1.1	>90
4	D	not done									
5	E	38.5	1.5	0.8	0.9	0.7	0.7	1.5	0.6	0.7	
6	F	not done									
7	G	165	13	5	6	3	5	17	0	2	>15
8	H	not done									
9	I	17.6	0.95-0.8	0.45	0.58	0.18	0.22	0.71	0.02	0.17	>1.5
10	J										
11	K										
12	L										

M. Abuzakouk:

Of the 13 laboratories involved 7 reported the above-mentioned results. All reported the strong EMA sample as AGA positive. Only one of the 7

reported the weak positive EMA as AGA positive. Two of the 7 reported the Sm antibodies positive sample as AGA positive.

IgA tTg

		Strong EMA	Weak EMA	pEMA+ nEMA-	ENA+	ANA+	Mit+	SM+	IgA-	normal	positive
	Centre/Sample	1	2	3	4	5	6	7	8	9	
1	A	not done									
2	B	32.2	11.6	4.8	3.8	4.3	2	10.4	0.1	1.5	>8.1
3	C	>25	4.6	1.3	1.6	1.1	1.6	3.6	0.12	0.61	>4
4	D	184	49	16	13	12	14	5	not done	8	>20
5	E	74	12	<3	<3	<3	<3	6	<3	<3	
6	F	122	30	9	7	4	2	11	0	1	>20
7	G	164	87	12	14	7	6	48	0	3	>25
8	H	not done									
9	I	95	250	74	86	60	708	75	70	27	>150
		29.7	9	4.7	4	3.6	1.5	9.8	0.01	1.4	>7
		42.6	4.7	<4	2	<4	<4	<4.5	<4	<4	>10
		>400	120	7	28	7.3	9	72	0.5	6	>28
10	J										
11	K										
12	L										

M. Abuzakouk:

The evaluation of the IgA tTG (see table above) was done in 10 of the 13 laboratories. All 10 labs reported the strong EMA+ sample as anti tTG positive. 9 out of 10 reported the weak EMA positive sample as an anti tTG positive. 4 out of 10 laboratories reported the Sm antibody positive sample as anti tTG positive. One of the 10 labs reported the anti Mit antibody positive sample as being anti tTg positive.

J. Schmitz:

What is the correlation between this series and the first results of the EMA tests? Was it the same laboratory, which found the false negatives?

M. Abuzakouk:

Yes

P. Collin:

Do you know what method was used for anti tTGA determinations?

M. Abuzakouk:

It was done according to the local protocol, but I do not have them here.

L. Mearin:

Different laboratories use different methods. We have the data at Leiden.

P. Collin:

It may be that the poor specificity depends on the use of guinea pig tTG, which was used in the older method.

Chr. Mulder:

Referring to the Sm antibody positive samples, they may be a sign of auto-immunity in CD as well as positivity for tTGA found in liver disease and thyroid disease. This illustrates the problem of tTGA specificity for CD.

P. Collin:

Maybe, but still there are many papers showing a specificity of almost 100%, which is difficult to believe.

C. Catassi:

What is the specificity of tTGA in these other diseases?

Chr. Mulder:

In those papers about thyroid patients the specificity is 60-70% only.

L. Mearin:

That was using human recombinant tTG?

Chr. Mulder:

Yes

P. Ciclitira:

That is very interesting. In America there is a strong movement to base the diagnosis of CD on tTGA results without biopsy. It is important that Europe reacts against it with scientific data.

C. Feighery:

We have the same experience in our extensive data on tTGA essays. When looking in sera from patients with connective tissue diseases we find tTGA frequently in those non-coeliac autoimmune individuals.

C. Catassi:

You probably are aware of a paper that we published coming from the Italian group of the NHL study. We found two patients with NHL; IgA positive tTGA and they were not coeliacs as showed by their biopsy and HLA. They were really false positive results. Should we have used the tTGA in our study I think we would have been fully amiss.

L. Mearin:

But even using EMA the diagnosis of CD should not only be serological, because e.g. in families where one child has CD other children may as well have positive EMA, but with normal small bowel biopsies. These “auto-immune families”, as I call them, make antibodies but they do not necessarily have enteropathy.

C. Feighery:

I think this study shows that there is very good concordance with strong EMA positivity and anti tTGA. Going back to the business of subjectivity with weak positive EMA you do see some level on anti tTGA activity in virtually the whole lot (9 out of 10). So from the point of view of sensitivity it is breaking out well. Specificity is where it falls down.

P. Collin:

We have also seen that many tTGA positive patients do not have DQ2 or DQ8.

F. Stevens:

Are the tTGA levels in these people with autoimmune disease as high as in coeliac patients?

C. Feighery:

No

F. Stevens:

So if you change the cut-off?

C. Feighery:

If you raise the cut-off you loose sensitivity.

M. Abuzakouk:

In conclusion there is a general agreement on the 9 different serum samples that were examined. Furthermore this small project of quality control enforced the current believe that EMA is the serological standard for the screening of CD.

L. Mearin:

If I understood correctly for the strong positive there was 100% agreement.

M. Abuzakouk:

Yes and for the weak positives 90%. The negative samples were all negative. But there was one lab that reported the Sm positive sample as an EMA positive. So we have one false positive.

J. Schmitz:

Was the weak EMA positive serum sample from a real coeliac patient? Was the mucosa also really flat or partial villous atrophy?

M. Abuzakouk:

It was partial villous atrophy.

J. Schmitz:

Does the weak EMA positive have the same value as the strong positivity?

M. Abuzakouk:

Most strong EMA positives are coeliacs. This patient was newly diagnosed and put on a diet.

C. Feighery:

We made a table for all the laboratories that took part in the tests and we will give you a copy with the code of you own laboratory. There was one laboratory that did 4 different anti tTG essays and the results were not necessary concordant between the different essays. So that was an interesting finding as well

L. Mearin:

You could publish the results of this quality control as an additional study. For the group it is important to see that we can rely on the EMA results of the different laboratory in the study.

C. Feighery:

The results are excellent.

C. Catassi:

It's worthy of a publication.

Chr. Mulder:

I think it would be difficult to publish. Your message is that between 12 laboratories the EMA concordance is quite good. That is what can be expected between these labs. It is not standardised for tTG methods between the labs.

L. Mearin:

I do not agree. I think that the message is important. If you are doing a Multicentre study in CD and you have to choose a serological screen test, choose the EMA.

Chr. Mulder:

But for future collaborative work you have to define all the things before such a study like cut-off points and EMA, which you standardise as much as possible between the labs.

C. Farré:

When you have a weak positive EMA and the patient does not have any gastroenterology symptoms: what do you do?

P. Collin:

A small bowel biopsy.

C. Farré:

An if the biopsy shows a little intraepithelial lymphocytosis and minimal lesions in the mucosa: What do you do?

P. Collin:

You have to consider the whole case. It depends on many things to decide whether the patient has CD or not. It is the job of the clinician then.

L. Mearin:

If I understand correctly Pekka means that CD not only is a serological diagnosis, not even a histological one, but a combination of genetic, immunologic, histological and clinical characteristics forming the "coeliac syndrome".

C. Farré:

Should I put a child with short stature and no other complaints, but EMA positive and minimal histological changes in the small bowel biopsy on a GFD?

L. Mearin:

We have a couple of patients in that position and I give them a try of GFD for about 1 year. I will tell them that I am not sure if they have CD, but that if they are growing under their target height, it is worth to try a GFD.

Chr. Mulder:

We will soon publish a paper in the Journal of Gastroenterology about 30-40 patients with minimal small bowel lesions after gluten challenge for diagnosis. Approximately 37 developed villous atrophy, but what to do with the others? More and more we give them GFD for 1 year. We try to do immune pheno-typing, DQ-typing and all that sort of thing. And then altogether give them advice. But if you only rely on histology or if you only rely on serology you have a problem. More and more you should work together with other disciplines to have a better answer.

Chair: C. Farré

**IEL quantification in small
bowel biopsies of patients
with NHL & CD**

N. Brousse

I will summarise the results of the cases with NHL and CD.

The cases from The Netherlands with recognized CD before the study:

UIC1013009 CD 15 years before EATL

UIC1013026 CD 5 years before EATL

UIC1013012 CD 3 years before EATL

UIC1011307 CD 7 years before diffuse large B cell of the lymph nodes.

These cases are clear-cut, both for CD as for the NHL. So I think we do not need to review them again now, but it is necessary to get all the precise clinical information, including data on the diet, and add this to the histopathology report.

The 3 cases from The Netherlands with unrecognised CD found through the study:

UIC1013008. The diffuse large B cell lymphoma is a classical one. As the patient has an autoimmune skin disease it is better to review the intestinal biopsy to be sure that is CD.

Discussion:

L. Mearin:

Even though the first 4 cases are known CD cases before the study I would suggest that the diagnostic small bowel biopsies would be sent to you as well. Actually, we should send you the biopsies of all these 17 or 18 cases with NHL and CD to be very sure about them. What do you think about it?

C. Catassi:

It is possible, but it may be difficult if the biopsy is from 15 years before, for example.

L. Mearin:

Sometimes it is difficult, but what I mean is that we should make an effort to try to get as many slides as possible and to send them to one central pathologist. Do we need to vote? Everybody agrees to send slides to N. Brousse in Paris.

UIC1011114 is a classical case of EATL. As criteria for CD there is partial villous atrophy of the small bowel mucosa with a strong staining with CD3 antibody, indicating intraepithelial lymphocytic infiltration. There is a small increase in de lamina propria as well. This phenotype is characteristic of CD.

Discussion:

C. Catassi:

With this case you not only stain the intraepithelial lymphocytes but also the lamina propria lymphocytes.

N. Brousse:

Yes all the CD3+ lymphocytes. You can use this antibody at any time even when the biopsy is as old as 20 years, if it is a paraffin block. This is not the case for not appropriately stained slides: the antibody will not stick on the glass, because we have to heat the slide for immunohistochemistry. So either you send me the paraffin blocks or special super frost slides for immunohistochemistry.

P. Ciclitira:

Do you microwave the sections?

N. Brousse;

Yes.

L. Mearin:

So the bottom-line of this patient is that you say that it is partial villous atrophy, but it is characteristic of CD.

UIC1010047 is an EATL with a special phenotype that is CD30+ and not CD3. I have the slides and the paraffin blocks. The EATL was simultaneously diagnosed with the CD. It is a classical diffuse large cell lymphoma. Here you can see that in the non-tumoral epithelium there are a lot of intraepithelial CD3 lymphocytes. They are CD8 positive: CD8 is negative on the tumour cells. CD8 is positive on the intraepithelial lymphocytes, which are extremely numerous. CD30 is a phenotype for activated cells. The intraepithelial lymphocytes are CD30 negative but the tumour cells are CD30+. That is called anaplastic large cell lymphoma: by molecular biology we can prove its T cell origin, but not with immunohistochemistry.

J. Schmitz:

Has it been demonstrated for this case?

N. Brousse:

I do not know.

Chr. Mulder:

Maybe it is helpful also to colour it with CD103 that shows mucosal origin.

N. Brousse:

That is right, but CD103 is only working on frozen sections and I have only a paraffin block.

L. Mearin:

We have considered this patient as an EATL, but should we have the molecular study?

N. Brousse:

No, it is clearly an EATL with a special activated phenotype. We see activated cytotoxic cells that express bronzyne B that is a phenotype of the intraepithelial lymphocytes.

L. Mearin:

So we do not want to do unnecessary work.

N. Brousse:

Except if you have the material we would be pleased to do the molecular study of the non-tumoral and tumoral tissue.

Chr. Mulder:

But this case is very suggestive to be of T cell origin because of the CD30 expression of probably mutating cells that have lost their ability to express CD3.

N. Brousse:

There is bronzyne B expression, a cytotoxic phenotype, which is characteristic for EATLs.

J. Schmitz:

The phenotype expressing bronzyne B is specific for intraepithelial lymphocytes in general, or CD8?

N. Brousse:

Intraepithelial lymphocytes are mostly CD8 and when then are more active they express bronzyne.

J. Schmitz:

So this is a good indication that this indeed is a T cell lymphoma.

N. Brousse:

Yes,

C. Feighery:

It could be a NK cell lymphoma. We need to be absolutely certain, and if we are not, we have to do further studies.

J. Schmitz:

But that depends upon the availability of the material. If we have it we should do it.

L. Mearin:

Even if it takes a bit of effort it is worth to do it.

N. Brousse:

Here we have tissue just around the tumour. It was CD53+: no villous atrophy but a very large increase of IEL.

P. Ciclitira:

If you saw that biopsy alone without the tumour: what would you say?

N. Brousse:

I would say to the physician please take more biopsies, because it must be something more than CD, such as refractory sprue. The mucosa is very ill.

L. Mearin:

I am trying to learn as well, but why is the mucosa so ill?

N. Brousse:

Normally there are not so many lymphocytes in CD.

M. Abuzakouk:

What about the lamina propria?

N. Brousse:

The lamina propria is not so affected. In this well orientated section there is no villous atrophy.

Chr. Mulder:

In my experience in family studies when you do biopsies on a low threshold, not waiting for EMA positivity, you can see quite regularly these sorts of alterations. Also in children with teeth abnormalities.

J. Schweizer:

Do you have duodenum biopsies?

N. Brousse:

These are surgical specimen taken from the jejunum.

P. Collin:

There maybe much more severe atrophy at proximal level.

N. Brousse:

In our experience with refractory sprue we see from stomach to the rectum this huge increase of IEL.

L. Mearin:

This lady is EATL and CD?

N. Brousse:

Yes.

L. Mearin:

The pathological description of these malignancies in CD should be published in a separate paper. Are you ready to do that part of the study?

N. Brousse:

That is my work.

L. Mearin:

There is the serological study that Mohamed was presenting and now this one. This is giving a lot of information and if we make an effort to get that material we can contribute with it.

C. Catassi:

It may be one publication with clinical and histological data.

Case from Italy

UIC1029001

The Italian case is clear-cut: CD was known for a long time and it is a classical B cell lymphoma.

Cases from Derby, UK

UIC103001

This is a classical case of CD 4-5 years before the study and a large B cell lymphoma.

UIC1030045

This case has to be commented. CD was recognised before the study and the T cell lymphoma arose in the skin. It should be studied if it is related to CD, because the most frequent site of extra-intestinal T cell lymphoma in CD is in the skin. We will have to find out if there is a relationship between the intestinal lymphocytes and the skin T cell lymphocytes.

C. Catassi:

Do you mean that the primary site could be in the gut?

N. Brousse:

Yes. So if we can have an actual small bowel biopsy we can study this. Skin lymphomas are epidermotropic, such as mycosis fungoides. I would need

frozen material to study if the lymphocytes are the same molecular population in the skin and in the gut.

Case from Sweden

UIC1040103

This case must be studied because we know that CD was recognised before the study, but we do not know anything about the lymphoma.

C. Hallert:

The patient refused a new small bowel biopsy.

N. Brousse:

We should at least know the primary site and the kind of lymphoma.

Cases from Dublin, Ireland

UIC1060002

CD was diagnosed in 1995. A B cell lymphoma in the omentum.

UIC1060016

CD was diagnosed in 1977. A B cell lymphoma in the tonsil.

UIC1060065

We discussed this case yesterday and we have to make sure that it is indeed CD and to review the skin lymphoma too, because an angiotrophic is a very unusual skin lymphoma.

M. Abuzakouk:

So you need the biopsies.

L. Mearin:

It is important to make sure that this patient is a coeliac. But more than the small bowel biopsies we do not have because the patient died.

N. Brousse:

Perhaps we can get information about what kind of lymphomas are common in dermatomyositis. Did the patient still have dermatomyositis when he developed the skin lymphoma?

L. Mearin:

I am afraid we have to take a decision about including this patient or not.

P. Collin:

It is clear that the patient had CD: the diagnosis has been established in Dublin and the patient was EMA positive.

C. Catassi:

It would probably be necessary to distinguish sure cases of CD and probable cases of CD and this decision should be taken in the next months.

J. Schmitz:

But in this case it is not so clear. As Con said EMA are not very specific in cases of autoimmunity.

P. Collin:

It was tTGA+, but not EMA+.

C. Catassi:

We should decide as well whether we include the 3 cases with refractory sprue or not.

M. Abuzakouk:

What more do we need? The patient had small bowel changes consistent with CD.

L. Mearin:

The small bowel changes were not typical for CD.

P. Collin:

We could make two analyses: one with the certain coeliacs and another one with the probable coeliacs.

R. Brand:

The CD status should be established 15th September 2001.

C. Catassi:

We would probably also ask to have information on the controls. We could discuss again the diagnosis of CD in the control group to do it in a symmetrical way with the cases.

L. Mearin:

The controls are clear-cut. Most of them. When there was not a biopsy available they were not considered as a coeliac.

Finland

UIC1050035

This case is not a problem. It is recognised CD since 1987 and an EALT. We have to review the phenotype, because it can be both CD4 and CD68, which recognises no T cells but histiocytes.

Galway, Ireland

UIC2060024

This is a clear-cut case of recognised CD since 1991 and an EATL. I have the slides, but not the special ones to do a good phenotyping.

Barcelona, Spain

UIC2080002

This case was diagnosed with CD 15 years before the lymphoma. A B cell large type lymphoma of the lymph nodes. It is a clear-cut case and does not need to be reviewed.

L. Mearin:

Nicole do you think it will be possible for you to put all these 17 or 18 cases in a kind of table and to say what you would wish to have as additional information. Then from the office in Leiden we could approach everybody and ask for it.

N. Brousse:

I will do that.

Chair: Carlo Catassi

HLA typing in patients with NHL and CD

J. Schweizer, Leiden

You can see the results in the table.

UIC	HLA-DQ
1010047	A0501 B0201
1011114	A0501 B0201
1011307	A0501 B0201
1013008	A0501 B0201/A0301 B0302
1013009	A0501 B0201
1013012	A0501 B0201
1013026	A0501 B0201
1029001	?
1030011	A0501 B0201
1030045	A0501 B0201
1040103	no material
	no material
1060002	no material
1060016	?
1060065	?
2060024	A0501 B0201
2080002	A0501 B0201
1110036	

I did not receive yet DNA from all the cases with NHL and CD, so please, if you have not sent DNA to Leiden, do it as soon as possible.

All the cases from The Netherlands are DQA0501 DQB0201, which is not a surprise.

The case from Italy gave some difficulty to our lab to pick up DNA from one of the cell pallets. But I have 3 more cell pallets.

The 2 cases from Derby are DQ2. I have not received DNA from Sweden, but I hope to get it soon.

There is a new case from Finland.

P. Collin:

I will send it next Monday or Tuesday.

J. Schweizer:

3 cases from Ireland: From one we will not be able to get any material. The two question marks for the other cases mean that I had problems taking DNA from one of the cell pallets, but we will try again. We extracted DNA from the paraffin block last week and I hope it will work, because sometimes it is too fragmented.

The cases from Galway and Barcelona are both DQ2.

There are 3 publications on HLA in coeliac patients who developed cancer. There was no difference in expression of HLA in the cancer patients in comparison to the other coeliacs. The frequency of some DRB haplotypes was different in the cancer group and maybe we should look at them as well.

J. Schmitz:

As we heard yesterday that most of these patients were following very correctly their GFD even up to 15 years it means that not only the gluten consumption plays a role in the occurrence of cancer. But what are the candidate genes, or protecting factors?

J. Schweizer:

There are interesting developments, such as information on the FAS genes, which play a role in the coeliac lesion and are mutated in lymphoma patients.

L. Mearin:

One of the ideas was to compare the genome of the patients with CD and NHL with the genome of the other coeliac patients. Perhaps taking up contact with one of the groups working on the coeliac genome. Did you approach the group in Utrecht?

J. Schweizer:

They got a bit delayed in collecting their families but they are ready now. They are trying to find candidate regions. As far as I know they have come up with MIC-B and are looking for it in the EATL patients of Chris.

C. Catassi:

Maybe some centre has information about the HLA of the lymphomas.

L. Mearin:

Perhaps there is some information in the literature.

J. Schweizer:

I have 20 DNA samples of lymphoma patients.

P. Collin:

But, what is the hypothesis?

C. Feighery:

The result is rather predictable, because we expect most coeliacs to be DQ2 positive, as the results presented in this table. But to extent the HLA study to the non-coeliac patients with NHL does not seem to have any advantage.

P. Collin:

I have a proposal. If any of us is working together with genetic expert, maybe we ask if we could do something with this material and if it makes sense to study these 18 cases.

C. Feighery:

If we have through hard endeavour collected all these samples we should not just hand them over to someone else.

F. Stevens:

Are there any intestinal T cell lymphomas that have been EMA negative and did not have a biopsy, because they should be looked up?

C. Catassi:

There are.

F. Stevens:

So they should be type DQ2.

L. Mearin:

We can have a look at them and to get as much information as possible about them. The HLA typing is more as a quality control of the diagnosis of CD in those cases.

Preliminary analysis:

Ronald Brand

I would like to discuss how I am going to analyse the data when they are complete. I will show some results but they are based on the data currently in the database and that could change later.

The matrix (the structure = case/control age and gender) of the data should be ready now and I need your signature that the matrix is correct. By September 15 the matrix will be filled in with the CD data and the EMA data.

If we look at the database we have 12000 records. Janneke Reehuis classifies the database for administrative purposes. We study partner by partner and we check if some item is missing. There is one category of cases with an initial entry, but because of protocol violation they can not enter the analysis.

E.g. if no informed consent was given, because the patient died. However, we can use the patient's data for scientific studies without consent as long as the patient remains anonymous.

Then we have the groups of controls that were handed in on overview tables (Sweden, Ireland, France and the Netherlands) instead of on individual basis. So I will produce a table with controls and cases. The table will first be split by gender and age, because we want to do a stratified analysis. An analysis with just the relationship between NHL and CD adjusted for possible differences in male/female ratio and age. Male/female poses no problem at all in the analysis, but age does. You can see on the table that there are countries where the ages between cases and controls are almost not overlapping and countries where there is overlap. We will make as well tables split up by CD status and perhaps by EMA positivity.

Generally a Mantel Haenszel analysis is used to calculate the relative risk (RR) in each country in each stratum for the age (e.g. 10 years) and then average over the strata. This means that a stratum, which contains only cases and no controls, does not contribute to the analysis. So we would lose cases if we simply use the stratified analysis.

Never calculate the RR for a case/control study, because it is an invalid measure. It is the ODDS ratio that should be calculated, which only can be done if there is at least 1 positive case (CD) among the NHL patients, or among the controls. So Italy, Derby Sweden, Spain and Ireland have an individual ODDS ratio. Those without an individual ODDS ratio contribute to the overall ODDS ratio. E.g. Italy has an ODDS ratio at this moment of 0.6 based on 3500 cases and controls, with a wide confidence interval. You can see that we have quite a lot of heterogeneity. We have a decreased risk in Italy and Spain, an increased risk in Derby, Sweden and Ireland. But if we test the homogeneity in Europe the results show that there is no reason to believe that the differences found are due to anything but to chance. If we repeat the whole Biomed project we will have a chance of 1 out of 3 to get the same variability between the countries. Then we get the common ODDS ratio which is the same as a Meta analysis, where partners are translated into publications and then you have a common ODDS ratio of 2-fold and a confidence interval from 0.9-4.

C. Catassi:

What do you mean with EMA positivity?

R. Brand:

This is ignoring any information about CD even if the CD status is unknown, and only using the EMA results.

L. Mearin:

It is not logical to analyse according to EMA positivity. If we do it like that we will ignore all the recognised coeliacs before the study.

P. Collin:

Yes, but the EMA positivity analysis gives another kind of information about the benefit of screening. Do we find silent coeliac cases with NHL if we take EMA positivity? With an ODDS ratio of 1 the answer is no.

L. Mearin:

Most of the cases with CD and NHL were previously recognised CD.

P. Collin:

Yes, but then screening NHL patients with a lymphoma for CD gives no additional benefit.

R. Brand:

I will analyse the results by conditional logistic regression, which predicts the probability of something. The conditional part refers to the fact that the partners will be analysed separately, like in the Mantel Haenszel method, but the effect of age and sex is established on the whole population instead of on the stratified one. First I analyse the relation between the probability of the outcome based on age and sex in the combined European population and then I adjust it for every partner. Sex seems to be highly significant in this study as the risk for females seems twice as much. I try to make you formulate the right questions, because there is still no clear-cut summary of the analysis that I can perform. You should compile a list of items that should be analysed before October 15. The information that we will get depends on the well-formulated questions you have asked and I can analyse. For example, it has been suggested to analyse if the relationship with CD is stronger within the T cell lymphoma than with the B cell lymphoma.

C. Catassi:

We should analyse as well the risk for CD in gut lymphoma altogether.

L. Mearin:

Risk analysis for skin lymphoma might be interesting too.

C. Feighery:

Risk in DH? The numbers will be small anyhow.

C. Catassi:

These are important questions because perhaps there is an increased risk for CD in some subgroups of NHL.

J. Schweizer:

We did the analysis of T cell lymphoma versus B cell lymphoma.

is in a group of 128 small bowel lymphomas. We found an ODDS ratio for CD of 73 for the T cell ones.

C. Feighery:

We might be able to come up with clinical questions to be analysed, but Ronald, you know the data well and you may come up with questions as well.

R. Brand:

I will, but from a methodological point of view. Since there are so many variables you should not exhaust the data. With only 17-18 cases with NHL and CD we can only use one or two co-variants in the analysis. Otherwise we will over-fit our model and will predict something that may be true in the Biomed study but not in other groups of patients.

J. Schmitz:

What does the ODDS ratio of 2 mean between the coeliacs and the controls?

R. Brand:

It means that of 2 persons with the same gender and age from the same country, one with NHL and the other not, the chance is a 2-fold increase to have CD for the first person in comparison to the chance of the other one. With the data that we have now the risk will not be more than a 5-fold increase. So, if you would say as a treating physician "I will not screen for CD unless there is a 10-fold increased chance", then this study shows that you should not screen for CD in NHL.

H. Szajewska:

Why can we not calculate the RR?

R. Brand:

In a case-control setting the researcher determines the number of cases and the number of controls by design. So the researcher can change the RR just by taking more controls or more cases. The ODDS ratio is the only measure that is independent of the sampling design of the study. That is the reason why statisticians and epidemiologists want ODDS ratios and not the more intuitive RR or the more appropriate risk (appropriate from the point of view of treatment).

Final meeting Paris

J. Schmitz

As part of the Organising Committee of the 10th International Symposium on Coeliac Disease, which will be held from Sunday 2 – 5 June 2002 at Paris, I can tell you that we are very happy to combine it with the last meeting of our Biomed project. The congress will take place at the new hall of the Pasteur Institute, which is very well adapted for this meeting. All the members of the organising committee are working at the Hôpital Necker – Enfants Malades in Pathology, Immunology, Gastroenterology and Paediatrics. The expected attendance, which will fit into the hall of the Pasteur institute, will be around 500 people. We will have a welcoming party on Sunday June 2 together with the registration. It will probably be a gala diner but certainly a faculty diner. The scientific program has been proposed by the organising committee and was sent for comments to a group of experts on CD. The title of the congress will be "Pathogenesis and Outcomes". In a few days from now we will decide the final program and send the invitations to the speakers. There will be a mixture of introductory lectures and selected presentations from the best abstracts. There will be posters probably presented during lunchtime and we are thinking of one or two satellite symposia.

Half of the meeting concerns pathogenesis, with the traditional topics. The morning of the first day will start with genetics. Then the role of tTG and auto-tTGA will follow. The day will end with the specific interaction of the immune cells in the mucosa. On Tuesday morning we will go on with the immune regulation, which is probably one of the most interesting topics, and with the intraepithelial lymphocytes. Tuesday afternoon starts with the clinical aspects and approaches at the two sides of the Atlantic in regard to the exploration of the coeliac iceberg. The end of the afternoon will be dedicated to the extra-intestinal manifestations. Wednesday morning will be for the screening. Then the malignant complications in which the Biomed Study may be reported. Then follow a few lectures on alternatives to the diet, which means futuristic treatment. And finally a few presentations interesting for the associations of patients about the diet, technically, sociologically and psychologically speaking. The meeting will end around 17.00-18.00 hours. Probably some people will have left in the afternoon to go to Taominia.

L. Mearin:

We have had a number of discussions on how we should present the results of this project, but we have to take a decision this afternoon. One possibility is to organise a meeting to present the results. As there are so many coeliac meetings I personally think that this is not a very good idea. Another possibility is to include our final meeting in the congress in Paris. Jacques had been so nice to tell us what the program will look like including the part on malignancy. I want to know what you think about it. Jacques put my name already in the preliminary program, but that is something we have to talk about.

J. Schmitz:

I would like to make a comment before the general discussion. We are going to obtain an important grant from the European Community, among other reasons also because we are including this Biomed study. During the congress the Biomed project must be present in one way or another. While I say that I realise that there is a very short time reserved for the presentation of the results of Biomed (09.40 – 11.10), so my proposition is to have in addition a Satellite Symposium on Biomed, for example Sunday afternoon.

H. Szajewska:

Maybe other option is to present the results at the ESPGHAN Taominia meeting, just after the Paris meeting.

C. Catassi:

ESPGHAN is a paediatric meeting.

L. Mearin:

It depends on what we want to do. The public of the Paris meeting will be coeliac freaks: 500 people really interested in CD, gastroenterologists,

immunologists, paediatric gastroenterologists etc. We will have a workshop in Taormina on CD and malignancy and there we will be able to give the message to the paediatric gastroenterologists.

C. Catassi:

This preliminary program seems quite nice to me. We could also present our results in another meeting e.g. next year during the European Gastroenterology Week.

Would it be possible to present the data at other meetings?

L. Mearin:

I find Paris ideal to present the results of our study because it is a coeliac congress with a very good timing for the project: The United European Week this year is too soon and next year too late.

H. Szajewska:

It is a good place but the question is when the results can be presented, on Sunday or on the last day of the meeting. I personally would be in favour of Sunday, because there is much more time and a lot of people will be there to take advantage of the cheaper flights on Saturday.

J. Schmitz:

I agree with Hania. This is a proposition I already made to Luisa. There can be a very good Biomed pre-meeting organised in the afternoon. And in addition it will be a strong formal statement in half an hour where the message will be given to the whole audience of the meeting.

H. Szajewska:

It would be very interesting to have this meeting. It could be open to everybody. The meeting could start with a Biomed symposium on malignancy on Sunday afternoon.

P. Collin:

Are there any other satellite symposia that Sunday?

J. Schmitz:

Until now there are no satellite meetings organised. If we could decide now that we have 3 hours for malignancy on Sunday afternoon it is OK.

L. Mearin:

We will be free to prepare our own program.

J. Schmitz:

Yes

L. Mearin:

What will be the financial consequences if we do it like that?

J. Schmitz:

I have not thought about that part. We have rented the room from lunchtime on Sunday onwards.

C. Hallert:

I think to start on Sunday afternoon would be fine.

L. Mearin:

When are you planning to send the invitations?

J. Schmitz:

Very soon. There will be preliminary announcements for the speakers sent in 10 days from now with the definite flyer to remind everyone about the days and what will happen. If you agree with what Hania said it will be put in the flyer, that there will be a pre-coeliac symposium concerning coeliac disease and malignancy under the Biomed responsibility so that people can organise their time. The final call for abstracts will be in October and then again people will be reminded again and if you want to put the program of the Biomed with this call for abstracts that is up to you.

L. Mearin:

What do you think Carlo?

C. Catassi:

I think that we have to respect the proposal of the Paris Organising Committee.

L. Mearin:

You say you will be happy to have just that session during the big meeting and no satellite meeting.

C. Catassi:

From my point of view our activity should be mostly on the publishing. But for what we have to say to the society the space they give us is enough.

R. Brand:

I thought Paris got financial support from Brussels especially for this purpose.

J. Schmitz:

The people in Brussels were very happy that Biomed was included in our symposium and that was the great facilitator for us to get the money.

L. Mearin:

One of the things Brussels wants is a European congress where the Biomed results can be announced.

L. Mearin:

We will have to make a decision about that satellite symposium. We will or we will not have it. We should vote.

P. Collin:

Everybody agrees that we have a satellite symposium and half an hour for our presentation at the meeting.

L. Mearin:

But it is important to realise that we have no money to prepare it.

N. Brousse:

You will not need the money, because in our hospital we can find a room.

J. Schmitz:

It will most probably be at the Pasteur Institute on Sunday.

C. Catassi:

You have to calculate the cost of the speakers.

H. Szajewska:

As far as I understand if some money is left we can not use it next year because the money should be spend by the end of this year.

C. Catassi:

My point would be that if we still have some money we should spend it on the publication.

H. Szajewska:

What is the chance that you will ask for extra money? Because a few meetings ago I remember you thought it was likely to get extra money for the final meeting.

L. Mearin:

But in that case we would have to organise our own Biomed symposium.

H. Szajewska:

If we organise a satellite symposium on Sunday afternoon that would be a separate Biomed Meeting and for that you could apply for extra money.

L. Mearin:

But now we are not going to get it because the application to Brussels for the 10th International Symposium did include the Biomed study and that was one of the reasons why Paris got money from Brussels. So they will not give it a second time.

R. Brand:

What is the difference Luisa in terms of money?

L. Mearin:

We will not pay anything.

H. Szajewska:

But you would not pay any extra money even with the satellite symposium on Sunday.

J. Schmitz:

There will be some travel expenses.

H. Szajewska:

There will be travel expenses whether we come on Sunday or whether the symposium will be on Wednesday.

L. Mearin:

But we have to be sure that the finances to organise the Sunday pre-symposium on Biomed will covered.

L. Mearin: (after voting)

Most of us voted to have the satellite symposium Sunday afternoon.

J. Schmitz:

It would be nice if we could bill the travel expenses for some of the people here who would otherwise not be invited.

R. Brand:

Our budget is completely empty by the November meeting of this group?

L. Mearin:

Yes by the end of December. And we do not have enough money to publish a book. I will show you after tea. But it is important that we have decided that we will have a satellite symposium Sunday afternoon. The first announcement will have to go with the next flyer that will be sent in October. During the congress we will just have the presentation of the results.

C. Catassi:

Maybe we should ask the group whether we want more money on the satellite or on the publication.

R. Brand:

You need to cover the travel and hotel expenses for the whole group.

J. Schmitz:

But as Hania said, most people would be coming on Sunday anyway.

H. Szajewska:

On Saturday because of the cheaper flight tickets.

J. Schmitz:

So it would mean that those expenses should be paid for people who have not been invited. From among the people who are here there will not be very many who are not invited. So it means maybe 4-5 people.

C. Feighery:

We do not need a lot of money except for a few people who will not be attending otherwise. We should certainly be able to raise the funding for that. We do not need money for an extra night in Paris because we are all going to arrive on Sunday already. With regard to the issue of having a full session in the International Symposium on Coeliac Disease I think we should not because we like to discuss some details and argue among ourselves etc, which I think will not be appropriate in the main symposium. So it seems to me ideal that we have accomplished travel for virtually everyone and I do not think it is a big issue.

J. Schmitz:

I completely agree.

L. Mearin:

So we have agreed that we will have a satellite symposium on Sunday afternoon and that this symposium is going to be announced by the Organisation of the Symposium and that the program for it will be ready in October or November.

J. Schmitz:

This is perfectly agreeable for the organising group I would say. My only final comment is that, in the time allotted to Biomed in the big meeting, it would be nice to have instead of what we proposed: the results and eventually a presentation of those 17 cases, because they are so interesting.

L. Mearin:

We could exchange it for the lecture on the gluten free diet.

Final publication

C. Catassi

The idea is that we will produce a major paper with the results of the frequency of CD in NHL and also a secondary publication on the clinical and histological features of CD associated to NHL. Maria Luisa, Pekka, Geoffrey and myself discussed whether it would be worthwhile also to publish a book. Our group has grown up in these last years by interacting with each other and with other experts. We have an extensive experience that would be worthy of a major publication. In a book we could also analyse the data of each country by itself, to give room to everyone in the group to publish the results separately. Luisa will show us some estimates of the costs that we will have if we go into the publication of a book. Of course you will

see that they vary very much depending on the publisher. If we want a visible book, which would be more expensive, we could try to get some money not only from Biomed, but maybe from other institutions.

L. Mearin:

ESTIMATE COSTS PUBLISHING BOOK
Prices in EURO

	<u>IOS Press</u>	<u>Kluwer Academic Publishers</u>	
200-250 pages	Hardcover CRC	Hardcover CRC	Hardcover
Price per copy			97.50 =35%
300 copies			29,250.00
Price per copy	12.00 – 15.00	24.50	82,50 =45%
500 copies	6,000 – 7,500.00	12,250.00	41,250.00
	10 weeks		
	Diskettes + formatted CRC		
		34.00	
500 copies		17,000.00	
Production		6-9 months	
Online	possible with	yes	
	access control		
Advantages	Brussels		distribution

Once the book has been published none of the publishing companies have problems with on-line distribution. IOS-Press however, wants some access control. It was not clear if they meant only by password or in terms of paying money as well. So what do you think about a Biomed book.

C. Catassi:

The first thing we have to decide is whether we want a book or not.

C. Feighery:

I am not sure if we should publish a book, but I would certainly go along with the general view. Looking at the offers we have I would certainly discount Karger because they are so expensive.

M. Abuzakouk:

I would say first the publication and then maybe a book.

E. Fabiani:

I agree with the book because it will give us a good idea of the frequencies of NHL and CD.

H. Szajewska:

I would give priority to the publishing of a paper.

R. Brand:

I can not see any reason for a book, because it will give no additional credit to the publication. Later, we can always publish a book on CD-rom because it has been written down already. It will cost not more than 5 Euro per copy.

C. Farré:

I do not understand the exact difference between a publication of a paper and a book.

C. Catassi:

We want to focus on the primary publication as our target. The book, if we decide to do it, would include other contributions focusing on CD and cancer.

K. Kaukinen:

Basically it is an interesting idea, but do we have enough subjects to make a book? Somehow I prefer paper.

F. Stevens:

Papers first.

P. Collin:

How can we write 250 pages about malignant disease that would interest other people beside ourselves? An alternative could be to write a special issue of 'Seminars in Oncology', which is definitely shorter than a book, but easier and less expensive.

C. Hallert:

I support a scientific paper.

J. Schmitz:

I agree with the point of view of Brand, Kaukinen and Hallert. I think once we have written the main papers there will be no interest in having a book. If we want to have ancillary publications one possibility will be to have a supplement to a journal like Gastroenterology or the Journal of Pediatric Gastroenterology and Nutrition. But once the main papers have been published I think everything has been said.

L. Mearin:

We should publish the proceedings of the final symposium in the Journal of Pediatric Gastroenterology and Nutrition?

J. Schmitz:

That would be a way.

N. Brousse:

I favour the publication of papers more than of books.

J. Schweizer:

I am not sure. I think there is more to cancer and CD than just epidemiology, although the epidemiological question is now the main issue, because the study is an epidemiological one. But I also think that it would be a waste to exclude the things we learned on the way and we should also grab a chance to share that with other 'aficionados' like us. I do not really see why we can do one thing and not do the other. I think we should write a book but give it another message, or a wider message than we can give it in the papers.

L. Mearin:

Most of you say: papers first as a peer reviewed publication are much more valued than a book.

J. Schmitz:

Publishing a book is a considerable loss of time. As soon as the paper is published cancer and CD will be less interesting, because the paper will show that the association is rare.

L. Mearin:

Another thing is of course that we have Internet and we can publish it online.

R. Band:

We could just publish it ourselves.

L. Mearin:

Some people will have to play a leading role in writing those papers.

C. Catassi:

We have to establish authorship criteria. We had some discussion in the beginning. I think we really should discuss this maybe during the last meeting.

L. Mearin:

I wanted to propose to you that we should go to the next meeting, which is going to be a hard working one, with the first draft of methods and results of the paper. No introduction, no discussion but methods and results. Therefore I want to try to make a plan with you to see how it will be possible to achieve that.

The final (6th) working meeting: We have to finish the 31st of December 2001. We will have our last meeting about 1 month before. The next working meeting will be **16th and 17th November 2001.**

C. Farré: During those days it will not be possible to have the meeting in Barcelona.

R. Brand:

I would propose that the meeting will have no social part at all and that we also use the evenings to discuss details of the analyses. I only got one month to prepare it. I would favour to have a personal talk of an hour with every partner and discuss what has to happen in the remaining part of November and December.

N. Brousse:

Why do we not try to work out as much as possible before the meeting, by deadlines.

L. Mearin:

We may have the meeting in Brussels, which can be easily reached.

Another city might be Rotterdam as it is the cultural capital of Europe this year. We have to work hard before the meeting and we have to make a plan to come to that meeting with the methods and results. We might organise the discussions with each partner by telephonic conference and E-mail.

After the 15th of September, which is the absolute deadline to return the forms to Ronald Brand with the checked data and your signature, we will have a working session with every partner, the data manager and the statistician to go through the data to be able to write the results and the methods.

J. Schmitz:

We discussed this morning that there should be a small group to ascertain the diagnosis of the 17 cases. Should this happen before 15th September?

L. Mearin:

We could do it during a telephonic conference 1 or 2 weeks after 15th September. We have to make an agreement about who is going to take part in that conference. N. Brousse is a very important person, because of the pathology. Most of the time P. Collin, C. Catassi, G. Holmes and myself have been conferring and I would propose to go on with that and include N. Brousse. But if you do not agree please say so.

C. Feighery:

We trust you. If we disagree with your decisions we will let you know.

L. Mearin:

The proceedings of this meeting will be send to you from Leiden together with a timetable so that you know when you can expect the data manager, the statistician and myself to contact you to have a good look at your data and discuss that by telephone.

R. Brand:

August 15 is the deadline for the signature on the number of cases and controls their gender and ages.

September 15 is the matrix filled in with CD and EMA positivity.

On September 16 Janneke will start checking all the data and in the period until October 15 we will produce the same report, send it to you for your signature and at the very latest the last signature must be returned by that time. After that nothing can be changed to any aspect of the database.

L. Mearin:

In the beginning of November I want to send to all of you the first draft of the methods and the results. You will have to send it back with your comments to the office before the meeting in November. With those comments we will have a second version and that is what we will discuss during the meeting.

R. Brand:

October 15 is your deadline to propose to me all the questions to be answered.

So we have an appointment now on October 15 to start analysing the data.

Keep a week free for that.

L. Mearin:

If we work like that there is a possibility to we will go to the meeting with more of less clear ideas of what we are going to discuss, and have some time for interpretation as well.

Financial overview

L. Mearin

Project Period	EEG BMH4-CT98-3091 01-05-1998 / 01-05-2001	
Budget	EURO Total Project	EURO Realisation
I. Receipts		
a. EC	250.000	155.545
Total I.	250.000	155.545
II. Expenditure		
a. Personnel	93.000	79.823
b. Support	29.000	18.245
c. Mobility	91.000	52.790
Subtotal	213.000	150.858
Overhead	27.000	28.157
Total II	250.000	179.014
Balance (I-II)	0	-23.469
Rest Budget 31/04/2001		70.986

Here you see the financial overview of the 250.000 EURO that we get for the project. The realisation is the money we received already from Brussels. In our case we are sure that we will get all of it. There is a deficit at the moment of 23.469 EUROS, which we have spent but not yet received from Brussels. On 1st May EURO 70.986 could still be spent. This meeting and the next one are not yet included here.

On the following table you can see a specification of the breakdown of costs. There is still some money for the data manager. The statistician has until now not spent what was calculated. We will not use the money for the medical doctor but among other things for help in collecting data for those who needed it. The money for the final meeting will be partly used for our 6th working meeting and to continue until 31st December. The overhead was calculated to be less expensive but that is quite expensive in The Netherlands. Altogether we still have enough to get us through until the end of the year.

Specification of Breakdown costs

I. Personnel		
a. Data manager	47.000	40.894
b. Statistician	17.000	5.627
c. Programmer	5.000	
d. Medical doctor	24.000	6.943
Total I.	93.000	53.464
II. Exchange-mobility		
a. 5 Progress meeting 5 x 14,000 = 70,000	70.000	52.790
b. Final meeting	21.000	
Total II	91.000	52.790
III. Support		
a. PC-internet	17.000	10.551
b. Other	8.000	7.694
c. Exchange/store sera/DNA	4.000	
Total III	29.000	18.245
IV. Overheads		
a. Secretary	25.000	26.359
b. Other overheads	12.000	28.157
Total IV	37.000	54.515

Agreements

1. Every partner should check her or his data and see if it is according to the forms R. Brand has handed out. These forms should then be signed and returned to the office in Leiden.
2. After 15th September 2001 no changes can be made to the cases and the controls in the database.
3. A detailed clinical and histological history should be made for all the 17 or 18 cases of non-Hodgkin lymphoma with CD, which should be sent to the office in Leiden.
4. The slides of the diagnostic small bowel biopsy and the lymphoma of all the non-Hodgkin lymphoma cases with CD should be sent to N. Brousse in Paris. An effort should be made to get such slides if they are not already available. N. Brousse will make a table of the slides that are in her possession already and those that are missing and send it to Leiden to be distributed among the partners.
5. After the 15th September during a telephonic conference C. Catassi, P. Collin, G. Holmes, N Brousse and L. Mearin will make the definite decision about when a coeliac is a coeliac.
6. The presentations of this meeting will be sent by E-mail to the office in Leiden so that they can be included in the proceedings. The proceedings will be on the Web pages of our project.
7. In preparation of the publication we will have a verification of the results during October and in the next meeting we will have the second draft for the methods and results of the publication.
8. We decided not to publish a book but to have one main publication and a number of ancillary publications in scientific journals. Perhaps they might all be put on-line later on.
9. Information about the gluten free diet for the cases and controls will be collected according to the 'Holmes' checklist
10. We will present the results of the project in Paris during the 10th International Symposium on Coeliac Disease that will be held from 2 – 5 June 2002. We will have a satellite symposium a day before the start of the congress, on Sunday afternoon 2nd June 2002. This symposium will be announced by the Organisation of the Paris congress.