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Soluble B-cell activation marker of sCD27 and sCD30 and future risk of B-cell lymphomas: A nested case-control study and meta-analyses

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Cancer Epidemiology

Prediagnostic serum/plasma concentrations of B-cell activation markers have been associated with future risk of B-cell lymphomas (BCL) in HIV-infected patients and in the general population. Current evidence for the general population is however limited and relies on relatively small numbers of observations, especially for specific histologies. We carried out a nested case-control study, including 218 BCL and 218 matched controls, within two prospective cohorts, to investigate the association between plasma levels of soluble (s)CD27 and sCD30 and future risk of BCL, and main histologic subtypes separately. To expand the evidence further, we performed meta-analyses of the published data on these associations from prospective studies among the general population. Our study revealed a significant relationship between sCD30 concentration and BCL risk (OR = 0.86, 1.53, 1.76, for the 2nd–4th quartiles respectively, p trend = 0.01). Similar increased risks were observed for diffuse large B-cell lymphoma and follicular lymphoma. Analyses of sCD27 blood concentrations did not show significant associations with BCL, (OR = 0.90, 1.26, 1.65 for the 2nd–4th quartiles, respectively, p trend = 0.17), but significant associations were observed for chronic lymphocytic leukaemia and for the group of "other BCL" subtypes. Our findings involving sCD30 were confirmed within our meta-analyses of five prospective cohorts, while results were more heterogeneous for sCD27 with the exception of CLL which was found consistently in all studies. Data to date suggest that chronic B-cell stimulation might be an important mechanism involved in B-cell lymphomagenesis both in HIV-infected and in the general population.

Key words: lymphoma, sCD30, sCD27, meta-analyses, prospective study

Abbreviations: 95% CI: 95% confidence intervals; AIDS: acquired immunodeficiency syndrome; ATBC: alpha-tocopherol, beta-carotene cancer prevention study; BCL: B-cell lymphomas; CLL: chronic lymphocytic leukaemia; CLR: conditional logistic regression; DLBCL: diffuse large B-cell lymphoma; EPIC-Italy: the Italian European Prospective Investigation into Cancer and Nutrition cohort; FL: follicular lymphoma; HIV: human immunodeficiency virus; IQR: interquartile range; M: mean; MED: median; MM: multiple myeloma; NHL: non-Hodgkin lymphoma; NOS: not otherwise specified; NSHDS: the Northern Sweden health and disease study; OR: odds ratios; PLCO: the prostate, lung, colorectal and ovarian cancer screening trial; PLL: prolymphocytic leukaemia; q: quartile; SCHS: Singapore Chinese health study; SCS: Shanghai cohort study; SD: standard deviation; SLL: small lymphocytic lymphoma; SWHS: Shanghai women's health study; TNF: tumour necrosis factor; TtD: time-to-diagnose; VIP: the Västerbotten Intervention program; WHI OS: the women's health initiative observational study

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What's new?

Soluble forms of tumor necrosis factor receptors, such as sCD27 and sCD30, are markers of B cell activation that can be readily measured in the blood stream. In this case-control study combined with a meta-analysis of previous studies, the authors examined the relationship between the two markers and the risk to develop B cell malignancies, in the general population. Their findings support the notion that chronic B-cell activation is an important mechanism involved in B-cell lymphomagenesis, which could be clinically exploited for developing risk prediction models in the future.

The risk of developing B-cell lymphomas (BCL) is markedly increased in HIV/AIDS patients, particularly for aggressive subtypes such as diffuse large B-cell lymphoma (DLBCL).[1,2] Sustained B-cell activation characterized by lymphocyte proliferation, class switch recombination, and somatic hypermutation, is suspected to be an important mechanism contributing to the accumulation of genetic alterations, leading to lymphomagenesis.[3,4] Elevated blood levels of several B-cell activation markers including soluble (s)CD23, sCD27, sCD30, sCD44, and CXCL13 have been linked to subsequent development of AIDS-related BCL with varying degree of evidence.[3,5] Among the general population, the most explored B-cell activation markers to date are sCD27 and sCD30. Recent prospective studies on healthy subjects have provided evidence that increased blood levels of sCD27[6,8] and sCD30[6–11] are associated with increased risk of lymphoma. Replication of these results, as proposed here, would provide strong evidence for chronic sustained B-cell activation being a major contributor to lymphomagenesis in the general population.

Both sCD27 (TNFRSF7) and sCD30 (TNFRSF8), are members of the tumour necrosis factor (TNF) receptor superfamily, and play an important role in regulating cellular activity in subsets of T, B, and natural killer cells.[12,13] Higher levels of both sCD27 and sCD30 have been found in persons with immune system activation, such as those with autoimmune disorders, hepatitis B and C viruses, and HIV.[14–20]

In a previous pilot study nested within the Italian European Prospective Investigation into Cancer and Nutrition cohort (EPIC, Italy), we reported on the association between increased prediagnostic blood levels of sCD30 and future risk of BCL.[9] To improve and extend results, notably incorporating histological stratification of BCL cases, we extended our analyses in the present study to plasma measurement of sCD30, sCD27 among participants of two prospective cohort studies among the general population: EPIC-Italy based on follow-up data till 2010 and the Northern Sweden Health and Disease Study (NSHDS). External validation of our findings was sought for from a meta-analytical framework using published data on sCD27 and sCD30 and lymphoma risk from recently published prospective studies in the general population.

Material and Methods

Subjects and cohorts

In the period 1993 to 1998, EPIC Italy completed the recruitment of 47,749 volunteers aged 35–70 years in five different areas covered by cancer registries: Varese ($n = 12{,}083$), Turin (10,604), Florence (13,597), Ragusa (6,403), and Naples (5,062, women only).[21] Incident primary cancer was identified by automated linkages to cancer and mortality Registries, population offices of municipalities where participants reported to be residing, hospital discharge systems, and periodic personal contacts (in Naples). The EPIC study was approved by the review board of the International Agency for Research on Cancer and by all local institutes recruiting participants.

The NSHDS cohort contains three subcohorts, of which we here used samples only from the Västerbotten Intervention program (VIP).[22] The VIP cohort comprises 80,000 healthy individuals aged 40–60 who were recruited between 1985 and 2008. Incident cancers occurring among cohort members during the study period were identified by linkage with the Swedish Cancer Registry and the local Northern Sweden Cancer Registry. The NSHDS was approved by the local research ethics committee.

After providing informed consent, detailed information on demographic, medical and lifestyle habits using standardized questionnaires were collected and a blood sample was taken. For both cohorts, blood samples were processed and placed in cold storage (liquid N_2 in EPIC, Italy and $-80°C$ in NSHDS).

For each incident B-cell lymphoma case identified within the two cohorts between 1 and 17 years after recruitment, one random control was selected among all cohort members alive and free of cancer at the time of diagnosis of the index case matched by cohort, center, gender, date of blood collection (± 6 months), and age at recruitment (± 2.5 years). $>95\%$ of participants also had the same fasting status as their matched pair at time of blood collection. Information from the two studies was standardized and integrated into a single database. Lymphoma cases were classified into subtypes according to SEER ICD-O-3 morphology codes.[23]

A total of 228 incident cases were initially identified during follow-up; 81 from EPIC Italy and 147 from NSHDS. After further subtype characterisation eight cases were reclassified as Hodgkin's lymphoma ($n = 3$), T-cell lymphoma ($n = 2$) and unknown ($n = 3$) and excluded from the analysis along with their matched controls. Moreover, two cases without suitable control samples were excluded. In total, 80 case-control pairs from EPIC-Italy (of which 28 cases were previously analysed for sCD30) and 138 pairs from NSHDS were included in the current study.

Table 1. General characteristic of B-cell lymphoma (BCL) cases and matched controls

Baseline variable	Cases ($n = 218$)	Controls ($n = 218$)	p*
Cohort, *n* (%)			
EPIC-Italy	80 (36.7%)	80 (36.7%)	
NSHDS	138 (63.3%)	138 (63.3%)	
Sex			
Male, *n* (%)	102 (46.8%)	102 (46.8%)	
Female, *n* (%)	116 (53.2%)	116 (53.2%)	
Age[1]	54.54 (6.58)	54.62 (6.47)	0.76
Body mass index[1]	26.51 (3.97)	26.35 (4.22)	0.68
Alcohol at recruitment (g day^{-1})[1]	7.80 (14.20)	8.69 (14.79)	0.30
Smoking status, *n* (%)			0.95
Never smoker	116 (53.2%)	119 (54.6%)	
Former smoker	57 (26.2%)	56 (25.7%)	
Current smoker	45 (20.6%)	43 (19.7%)	
Unknown			
Physical activity, *n* (%)			0.39
Inactive	60 (27.5%)	61 (28.0%)	
Moderately inactive	85 (39.0%)	84 (38.5%)	
Moderately active	57 (26.2%)	51 (23.4%)	
Active	14 (6.4%)	22 (10.1%)	
Unknown	2 (0.9%)	0	
Education, *n* (%)			0.72
None	2 (0.9%)	1 (0.5%)	
Primary school	90 (41.3%)	82 (37.6%)	
Technical/professional school	57 (26.2%)	53 (24.3%)	
Secondary school	37 (17.0%)	46 (21.1%)	
Longer education (incl. university degree)	32 (14.7%)	36 (16.5%)	
BCL subtypes, *n* (%)			
Diffuse large B-cell lymphoma	38 (17.4%)		
Follicular lymphoma	38 (17.4%)		
Chronic lymphocytic leukaemia	35 (16.1%)		
Multiple myeloma	39 (17.9%)		
'Other subtypes'	68 (31.2%)		

Italian European Prospective Investigation into Cancer and Nutrition cohort (EPIC-Italy); Northern Sweden Health and Disease Study (NSHDS); *p values calculated using paired *t test* for continues and Chi- square test for categorical variables. [1]Mean (SD).

Plasma sCD27 and sCD30

Blood samples were collected by venipuncture in citrate (Italy) or EDTA (Sweden) plasma vacutainers and processed by centrifugation. Plasma level of sCD27 and sCD30 were measured by ELISA (Bender Medsystems: BMS286INST and BMS240 kits, respectively). All laboratory personnel were blinded with regard to case-control status. Median time interval between sample collection and freezing was ~1 hrs for both cases and controls. Matched case-control sets were assayed in the same batch and quality control samples were run in duplicate along with the case-control sets in each batch. All sCD27 measurements were done in duplicate. For sCD30, due to limited plasma volume, only a subset of samples were run in duplicate [$n = 151$ (34%)]. The median coefficient of variation was 5.10 and 2.60% and intra class of correlation coefficients was 0.95 and 0.99 for sCD27 and sCD30, respectively.

Data analysis

Blood levels of soluble markers were log transformed to normalize their distributions. Differences between cases and controls in baseline continuous covariates were assessed using

Table 2. Odds ratio (OR) and 95% confidence interval for sCD30 and sCD27 measurements and B-cell lymphoma (BCL) and histological subtypes

	Logistic regression[1]				
sCD30	**Q1 (1.84–2.99)**	**Q2 (3.00–3.16)**	**Q3 (3.17–3.41)**	**Q4 (3.42–5.56)**	***p***
BCL					
N (case/control)	45/54	37/55	63/55	73/54	
OR (95% CI)	Ref.	0.86 (0.48–1.54)	1.53 (0.87–2.69)	1.76 (1.01–3.07)	0.01
BCL without MM					
N	35/47	27/43	52/47	65/42	
OR (95% CI)	Ref.	0.92 (0.47–1.80)	1.75 (0.92–3.35)	2.30 (1.23–4.30)	0.002
DLBCL					
N	6/54	3/55	11/55	18/54	
OR (95% CI)	Ref.	0.52 (0.13–2.08)	1.74 (0.64–4.75)	2.57 (1.01–6.59)	0.01
FL					
N	5/54	11/55	9/55	13/54	
OR (95% CI)	Ref.	2.10 (0.72–6.18)	2.00 (0.63–6.31)	3.19 (1.10–9.23)	0.04
CLL					
N	9/54	6/55	10/55	10/54	
OR (95% CI)	Ref.	0.76 (0.26–2.24)	1.18 (0.46–3.04)	1.22 (0.47–3.16)	0.51
MM					
N	10/54	10/55	11/55	8/54	
OR (95% CI)	Ref.	0.87 (0.35–2.19)	0.95 (0.39–2.33)	0.67 (0.26–v1.73)	0.47
'*Other BCL*'					
N	15/54	7/55	22/55	24/54	
OR (95% CI)	Ref.	0.54 (0.22–1.34)	1.24 (0.63–2.45)	1.31 (0.68–2.55)	0.19
sCD27	**Q1 (0.56-3.44)**	**Q2 (3.45–3.92)**	**Q3 (3.93–4.35)**	**Q4 (4.36–6.50)**	
BCL					
N (case/control)	54/55	44/54	58/55	62/54	
OR (95% CI)	Ref.	0.90 (0.46–1.75)	1.26 (0.60–2.66)	1.65 (0.69–3.92)	0.17
BCL without MM					
N	45/46	32/45	52/50	50/38	
OR (95% CI)	Ref.	0.85 (0.40–1.81)	1.29 (0.57–2.91)	2.27 (0.85–6.05)	0.05
DLBCL					
N	11/55	8/54	9/55	10/54	
OR (95% CI)	Ref.	0.41 (0.10–1.60)	0.33 (0.07–1.55)	0.54 (0.11–2.77)	0.71
FL					
N	11/55	9/54	10/55	8/54	
OR (95% CI)	Ref.	0.87 (0.30–2.56)	0.80 (0.22–2.85)	1.11 (0.25–5.03)	0.94
CLL					
N	7/55	4/54	12/55	12/54	
OR (95% CI)	Ref.	0.71 (0.18–2.75)	2.38 (0.73–7.82)	3.85 (0.97–15.29)	0.02
MM					
N	9/55	4/54	6/55	12/54	
OR (95% CI)	Ref.	1.39 (0.37–5.21)	0.80 (0.16–3.89)	0.89 (0.14–5.49)	0.65
'*Other BCL*'					
N	16/55	11/54	21/55	20/54	
OR (95% CI)	Ref.	1.06 (0.41–2.73)	2.19 (0.87–5.52)	2.53 (0.84–7.65)	0.04

Quartile (Q); diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); chronic lymphocytic leukaemia (CLL); multiple myeloma (MM); Other BCL subtypes ('*Other BCL*'); Blood levels of immune markers were categorized into quartiles based on the distribution among controls; *p* trend = *p* values (two sided) were calculated by including the quartile number as continues variable. [1]Conditional logistic regression was used for total BCL and BCL without MM and unconditional logistic regression analysis additionally adjusted for age, sex, country, and plate number was used for subtypes.

Table 3. Multivariable logistic regression models for B-cell lymphoma (BCL) and quartiles (Q) of sCD30 and sCD27 concentrations stratified by time from blood collection to case diagnosis (TtD)

	Logistic regression				
sCD30	**Q1**	**Q2**	**Q3**	**Q4**	***P***
BCL excluding MM TtD≤6					
N (case/control)	16/54	14/55	25/55	34/54	
OR (95% CI)	Ref.	0.94 (0.45–1.94)	1.45 (0.76–2.77)	1.69 (0.92–3.11)	0.04
BCL excluding MM TtD>6					
N (case/control)	19/54	13/55	27/55	31/54	
OR (95% CI)	Ref.	0.81 (0.39–1.69)	1.47 (0.79–2.74)	1.62 (0.88–3.00)	0.05
sCD27					
BCL excluding MM TtD≤6					
N (case/control)	26/55	18/54	21/55	24/54	
OR (95% CI)	Ref.	0.72 (0.39–1.34)	0.78 (0.43–1.43)	1.04 (0.59–1.84)	0.93
BCL excluding MM TtD>6					
N (case/control)	19/55	14/54	31/55	26/54	
OR (95% CI)	Ref.	0.81 (0.39–1.66)	1.32 (0.72–2.42)	1.25 (0.68–2.31)	0.25

paired t test, and by χ^2 test, for categorical variables. Odds ratios (OR) and 95% confidence intervals (95% CI) for BCL in relation to soluble markers were calculated by conditional logistic regression (CLR) as parameterised in the PHREG procedure (SAS statistical software, version 9.1; SAS Institute).

Non-parametric investigation of the potential associations relied on the discretization of soluble markers concentrations, which were recoded in quartiles, calculated from control subjects. Potential confounding was investigated for body mass index (in kg m^{-2}; continuous), alcohol intake (g day^{-1}; continuous), smoking status (non-smoker, former smoker, smoker), Cambridge physical activity index (inactive, moderately inactive, moderately active, active) and educational level (none, primary, technical/professional, secondary, university/college). Potential confounders that yield a change in the risk estimates >10% were taken forward and included in the final models. Tests for trend were calculated using the quartile number as a continuous variable.

Analyses were done for all BCL cases together with or without inclusion of multiple myeloma and for each of the main histological subtypes separately. Further stratification included time from blood collection to diagnosis (below or above the median observation: 6 years) to explore the possibility of reverse causation. In these analyses, to preserve statistical power, subtype cases were compared to all controls. To appropriately accommodate the resulting unmatched design, we ran nonconditional logistic regression, adjusted for matching variables (*i.e.*, country, gender, and age at recruitment) and plate number. Statistical analyses were done using the R 3.0.1 language and environment (The R Foundation for Statistical Computing, Vienna, Austria) and SAS (version 9.1; SAS institute). All p values are two-sided, with $p < 0.05$ considered as statistically significant.

Meta-analysis

We conducted a search of PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez), web of science and EMBASE using the key words "sCD30" or "sCD27" and "lymphoma." We included publications in the meta-analysis if they were published in the peer-reviewed literature (English), reported results for non-Hodgkin lymphoma and/or on B-cell lymphoma subtypes [*i.e.*, DLBCL, follicular lymphoma (FL), chronic lymphocytic leukaemia (CLL), and multiple myeloma (MM)] and were conducted among the general population in a prospective setting. The meta-analysis was restricted to studies among the general population, and did not include studies among HIV+/AIDS infected populations, as to keep the source population similar to our study. We checked references in all identified publications for additional studies.

To allow the inclusion of studies with different categorization for the soluble markers in our meta-analysis, we pooled ORs by conducting a within-study random-effects meta-analysis of the nonreference categories of the soluble markers. This effectively provided a meta-risk estimate of all categories versus the study reference category. Subsequently, we used the random effects model to meta-analyse the studies. DerSimonian–Laird method was used as the random-effects model. The coefficient of inconsistency (I^2) was used as a metric to assess heterogeneity between studies, with a p values < 0.05 to be regarded as statistical significant evidence for between study heterogeneity. Meta-analyses were carried out using Comprehensive Meta-Analysis (CMA) software, version 2.

Results

Our study included 218 B-cell lymphoma cases and an equal number of controls (102 men and 116 women) (Table 1).

Table 4. Characteristics of included studies in the meta-analyses

	Purdue_2009	Purdue_2011	De Roos_2012	Purdue_2015	Bassig_2015	Present study[1]
Cohort	PLCO	PLCO	WHI OS	ATBC	SWHS+ SCS + SCHS	EPIC-Italy + NSHDS
Recruitment years	1993–2001	1993–2001	1994–1998	1985-1988	1996-2000,1986–1989, 1993-1998	1993–1998, 1990-2006
Follow-up, years	1–10	1–10	0–13	2-23	0 to >10	1–17
Case/Control (*n*)	234/234	297/297	491/491	272/325	218/218	179/179
Age	55–74	55–74	50–79	50-69	40–74	35–70
Gender	M/F	M/F	F	M	M/F	M/F
BCL subtypes (*n*)	CLL/SLL (97) DLBCL (52) FL (40) Other/NOS (45)	CLL/SLL (117) DLBCL (62) FL (44) Other/NOS (74)	CLL/SLL/PLL (142) DLBCL (138) FL (102) Other/NOS (109)	CLL/SLL (74) DLBCL (60) FL (25) Other/NOS (113)	-	CLL/SLL (35); DLBCL (38) FL (38) Other/NOS (68)
sCD30 level cases, ng mL^{-1}	MED (IQR): 18.8 (11.3-29.7)	–	–	–	MED (P25–P75): 38.4 (29.2-49.9)	M (SD): 30.5 (16.1) MED: 26.1
sCD30 level controls, ng mL^{-1}	MED (IQR): 13.5 (8.8-20.6)	–	–	–	MED (P25–P75): 31.4 (24.8-38.9)	M(SD): 28.6 (21.7) MED: 23.2
sCD30 categories	Q1: ≤8.8 Q2: 8.9–13.4 Q3: 13.5–20.6 Q4: >20.6 For subtypes: based on control's median; m1: <13.5 m2: ≥13.5	–	Q1: <24.1; Q2: 24.1–30.1; Q3: 30.2–40.1; Q4: ≥40.2	Q1: ≤19.3 Q2: 19.3–29.8; Q3: >29.8	Q1: ≤24.8; Q2: 24.8–31.5; Q3: 31.5–38.9; Q4: >38.9	Q1: ≤19.9; Q2: 20–23.5; Q3: 23.6–30.4; Q4: ≥30.5
sCD30 estimates for BCL	Q1 = ref. Q2 = 1.4 (0.8–2.6) Q3 = 2.2 (1.2–4.1) Q4 = 4.1 (2.2–7.8)	–	Q1 = ref.; Q2 = 1.4 (0.9–2); Q3 = 1.6(1–2.3); Q4 = 2.8 (1.9–4.2)	Q1 = ref.; Q2 = 2.5 (1.5–4.2); Q3 = 3.3 (1.9–5.5)	Q1 = ref.; Q2 = 1.74 (0.85–3.58); Q3 = 1.86 (0.94–3.67); Q4 = 5.15 (2.62–10.12)	Q1 = ref.; Q2 = 0.92 (0.47–1.80); Q3 = 1.75 (0.92–3.35); Q4 = 2.30 (1.23–4.30)
sCD27 level case, U mL^{-1}	–	75 (24.2, 163.9), MED (P5, P95)	–	–	MED (P25–P75): 107 (84–133)	M(SD): 69.13 (64.4); MED: 55.3
sCD27 level control, U mL^{-1}	–	MED (P5, P95): 58.7 (27.3, 108)	–	–	MED (P25–P75): 92 (73–115)	M (SD): 61.31 (44.6); MED: 50.7
sCD27categories	–	Q1: ≤47.82; Q2: 47.83-58.71; Q3: 58.72-72.73; Q4: >72.73; For subtypes: based on control's median; m1: <58.7; m2: ≥58.7	Q1: <177; Q2: 177–223.9; Q3: 224–274.9; Q4: ≥275	Q1: ≤44; Q2: 45–61; Q3: >61	Q1: ≤73; Q2: 73–91.5; Q3: 91.5–115; Q4: >115	Q1: ≤31.3; Q2: 31.4–50.6; Q3: 50.7–77; Q4: ≥78

Table 4. Characteristics of included studies in the meta-analyses (Continued)

	Purdue_2009	Purdue_2011	De Roos_2012	Purdue_2015	Bassig_2015	Present study[1]
sCD27 estimates for BCL	–	Q1 = ref.; Q2 = 0.9 (0.5–1.6); Q3 = 1.3 (0.8–2.3); Q4 = 5.3 (2.9–9.4)	Q1 = ref.; Q2 = 2.1 (1.4–3.4); Q3 = 2.8 (1.7–4.4); Q4 = 5.5 (3.5–8.7)	Q1 = ref.; Q2 = 2.0 (1.2–3.1); Q3 = 3.0 (1.8–4.9)	Q1 = ref.; Q2 = 1.60 (0.83–3.09); Q3 = 1.94 (0.98–3.83); Q4 = 4.45 (2.25–8.81)	Q1 = ref.; Q2 = 0.85 (0.40–1.81); Q3 = 1.29 (0.57–2.91); Q4 = 2.27 (0.85–6.05)

[1]Data is based on B-cell lymphoma (BCL) cases excluding multiple myeloma; Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO); Women's Health Initiative Observational Study (WHI OS); European Prospective Investigation into Cancer and Nutrition cohort (EPIC); Northern Sweden Health and Disease Study (NSHDS); Alpha-Tocopherol, Beta-Carotene Cancer Prevention study (ATBC); Shanghai Women's Health Study (SWHS); Shanghai Cohort Study (SCS); Singapore Chinese Health Study (SCHS); diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); chronic lymphocytic leukaemia (CLL); prolymphocytic leukaemia (PLL); small lymphocytic lymphoma (SLL); not otherwise specified (NOS); quartile (Q); mean (M); standard deviation (SD); median (MED); interquartile range (IQR).

Median time between blood collection and diagnosis of BCL was 6.2 years (range, 1–16). 17.4% of cases were diagnosed with DLBCL and FL, 17.9% with MM, 16.1% with CLL. Cases and control subjects did not differ with respect to established risk factors and covariates (Table 1).

Risk estimates

CLR analyses for all BCL cases showed a significant association for sCD30 (OR = 1.76, for 4thQ *vs.* 1stQ, p trend = 0.01) (Table 2). Elevated levels of sCD27 seemed to increase the risk of BCL, although the finding did not reach statistical significance (OR = 1.65, for 4thQ *vs.* 1stQ, p trend = 0.17). The analyses of BCL excluding MM cases showed similar results as total BCL; sCD30 (OR = 2.30, for 4thQ *vs.* 1stQ, p trend = 0.002) and sCD27 (OR = 2.27, for 4thQ *vs.* 1stQ, p trend = 0.05) (Table 2).

In subtype specific analyses (Table 2), increasing plasma levels of sCD30 were associated with risk of DLBCL (OR = 2.57, for 4thQ *vs.* 1stQ, p trend = 0.01) and FL (OR = 3.19, for 4thQ *vs.* 1stQ, p trend = 0.04) while sCD27 level was associated with increased risk of CLL (OR = 3.85, for 4thQ *vs.* 1stQ, p trend = 0.02) and "other BCL" subtypes (OR = 2.53, for 4thQ *vs.* 1stQ, p trend = 0.04). For both levels of sCD27 and sCD30, we did not find any statistically significant associations with risk of MM. Conditional analyses for histological subtypes rendered similar results albeit with wider confidence intervals due to the inclusion of only individually matched controls (data not shown).

Analyses stratified by time from blood collection to case diagnosis (time-to-diagnosis: TtD) showed that the association between sCD30 and BCL excluding MM was marginally affected by TtD (Table 3). Continuous analyses between plasma sCD30 concentrations and TtD among BCL cases excluding MM revealed however a significant trend ($p = 0.03$; Supporting Information Fig.) suggesting a possible influence of the disease itself on sCD30 plasma levels. Stratified analyses by TtD for sCD27 and BCL excluding MM did not reveal an influence of TtD on the observed (absence of) association. Moreover, continuous analyses among cases did not show a strong association between TtD and plasma sCD27 concentrations. Analyses of TtD within BCL sub-types were limited by the low case numbers but revealed some evidence that the association between sCD30 and DLBCL and FL were influenced by TtD with stronger associations in the sub-group with TtD $>$ 6 years while the results for CLL and sCD27 were stronger in the case group with a TtD $<$ 6 years before diagnosis (data not shown).

Meta-analyses results

Data were available from six original reports including two studies from the prostate, lung, colorectal, and ovarian cancer screening trial (PLCO),[7,10] one study nested within the Women's Health Initiative Observational Study (WHI OS) cohort,[6] one study nested within the Alpha-Tocopherol, Beta-

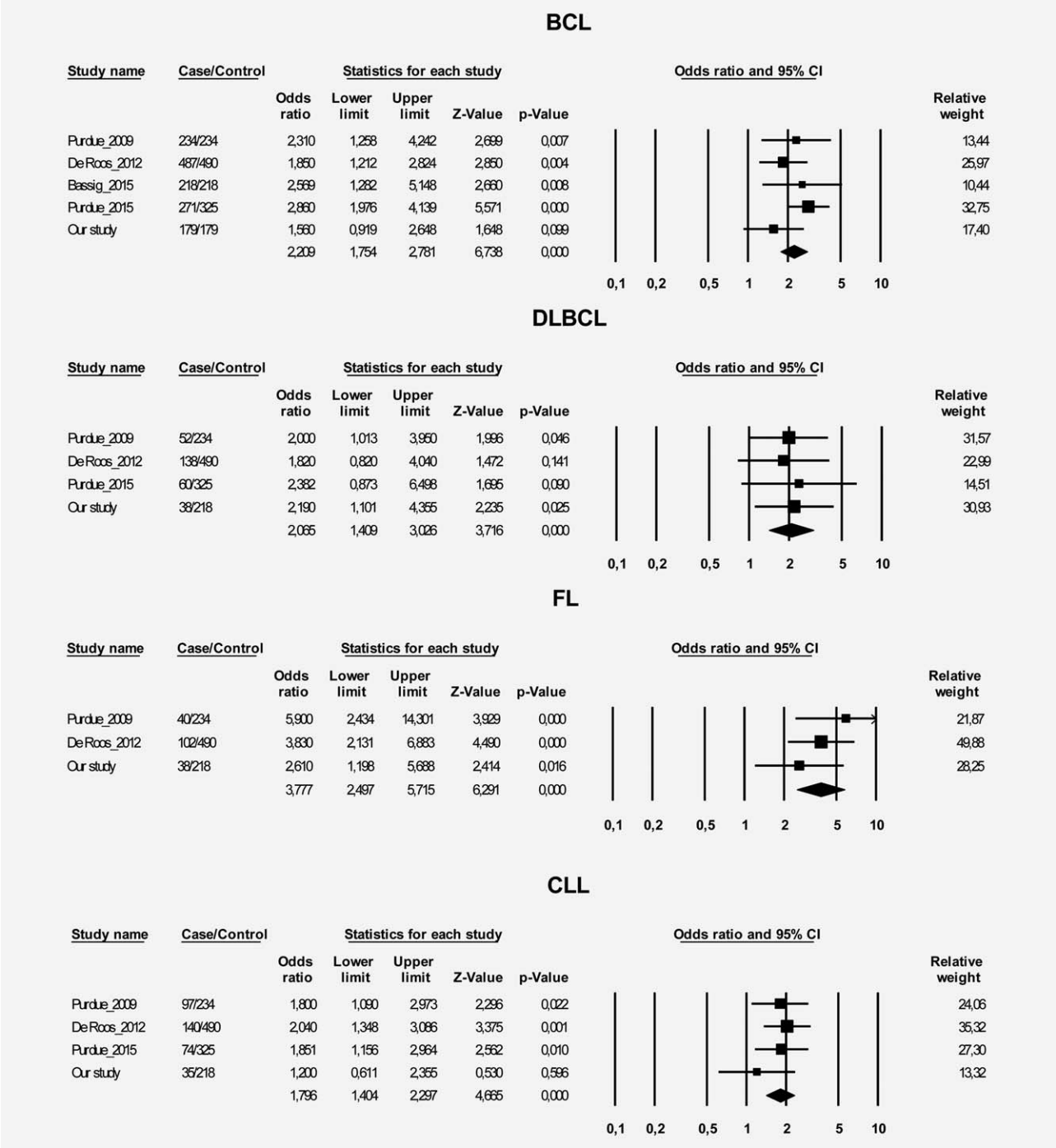

Figure 1. Meta-analyses (random effects model) of five prospective studies on the association between log-transformed blood levels of sCD30 and risk of B-cell lymphoma (BCL) and major histological subtypes in the general population; diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); chronic lymphocytic leukaemia (CLL); reported ORs for individual studies are based on a within-study random-effects meta-analysis of the non-reference categories of the soluble markers.

Carotene Cancer Prevention study (ATBC),[11] a nested case-control study within three Asian cohorts[8] including the Shanghai Women's Health Study (SWHS), Shanghai Cohort Study (SCS), and Singapore Chinese Health Study (SCHS), and one from the EPIC-Italy cohort.[9] Nearly 28 BCL cases from the EPIC-Italy cohort[9] were also included in current study. Therefore we excluded this study from our meta-analysis. As the case definition by the other cohorts did not include MM, the presented results for BCL excluding MM cases (Table 2) were included in our meta-analysis. Table 4 lists all publications that contributed to our meta-analysis. The meta-analyses of sCD30 showed highly consistent results

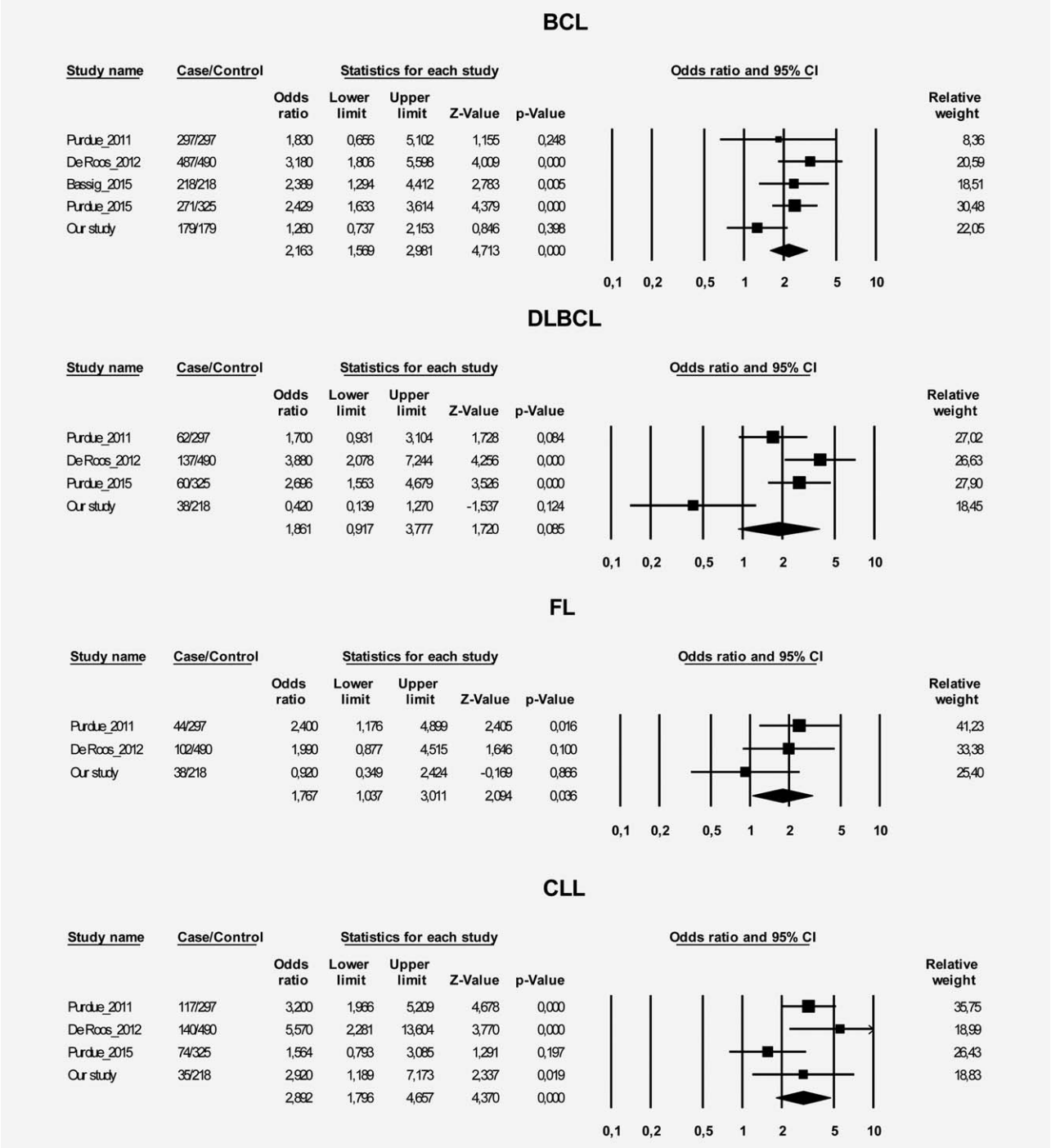

Figure 2. Meta-analyses (random effects model) of five prospective studies on the association between log-transformed blood levels of sCD27 and risk of B-cell lymphoma (BCL) and major histological subtypes in the general population; diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); chronic lymphocytic leukaemia (CLL); reported ORs for individual studies are based on a within-study random-effects meta-analysis of the non-reference categories of the soluble markers.

across prospective cohorts, in particular for the subtype specific analyses (Fig. 1; I^2 9.3% for BCL and 0% by subtypes) while there was some indication of heterogeneity across the studies for sCD27 with the BCL and subtypes (Fig. 2; I^2 35.7% FOR BCL; 20.8% FL, 76.9% DLBCL, and 44.2% for CLL).

Discussion

In this investigation of plasma levels of soluble markers and subsequent risk of BCL, elevated pre-diagnostic levels of sCD30 were significantly associated with increased risk of BCL, in particular DLBCL and FL. Moreover elevated levels of sCD27 were significantly associated with CLL; however,

the association seemed to weaken with increasing time-to-diagnosis. Because of small sample size of the stratified analyses by TtD, these results should be interpreted with caution.

We observed significant increased risks of BCL, DLBCL, and FL with elevated levels of sCD30, which are consistent with previous prospective cohort studies.[6,8,10,11] An increased risk of CLL and "other BCL" with sCD30 was reported by Purdue et al.,[10] while our results did not reach statistical significance for these subtypes. Taken together our meta-analysis provides increased support for an association of pre-diagnostic sCD30 levels with BCL and the major subtypes of DLBCL, FL, and CLL among immunocompetent people with no observed heterogeneity between studies. As such the association seems to be independent of gender and of ethnic background as results were similar between studies including only men (Purdue study) or women (Roos study) and between studies performed in Europe (EPIC, ATBC), US (PLCO, WHI), and East Asia (Bassig study). There are known differences in pathophysiological mechanisms involved in the development of BCL subtypes, our finding of sCD30 being associated to all subtypes may possibly reflect an underlying pathway, characterized by changes in sCD30 levels, that would be common across BCL histological subtypes. Studies have shown that CD30 is expressed by several types of T- and BCL, such as anaplastic large cell lymphoma, primary mediastinal large B-cell lymphoma, and Epstein–Barr virus driven clonal lymphoproliferative disorders[24] and correlates with prognosis.[25] Moreover, several immune-related disorders, such as atopy, HIV infection, infectious mononucleosis, chronic hepatitis B and C, systemic lupus erythematosus, bullous pemphigoid and Ommenn's syndrome, are characterized by increased serum sCD30 concentrations.[26–28] These data might suggest a link between immune modulators, changes in B-cell activation as represented by sCD30 and lymphoma risk. Although our analyses showed a significant inverse association between sCD30 levels and duration of TtD among DLBCL and FL cases, the stratified results by median TtD did not reveal a strong indication of reverse causation/disease-induced bias.

Consistently with previous studies,[6,7,11] our analyses showed a significant association between sCD27 level and risk of CLL and "other BCL." These studies also reported significant associations between higher levels of plasma sCD27 and increased risks of DLBCL,[6,11] and FL.[6,7] Our data did not provide additional support for these associations. Our meta-analyses suggest that sCD27 levels are associated with increased risk for BCL, FL, and CLL, but not for DLBCL. Meta-analysis of plasma levels of sCD27 in relation to risk of DLBCL showed marked heterogeneity between studies. The resulting inconsistency in findings may partly be explained by the different age distribution of study populations and different distributions of BCL subtypes which vary considerably from population to population, and could lead to some variation in reported associations in particular for total BCL. Expression of CD27 is restricted to cells of the lymphoid lineage and appears to be related to functional differentiation of T- and B-cells.[29] CD27 receptor, upon activation by its ligand CD70, stimulates T-cell proliferation and enhances immunoglobulin production in B cells.[7] Elevated levels of soluble CD27, cleaved from CD27-positive cells, have been observed in many infectious and autoimmune diseases and have been proposed as a marker of immune activation.[12] Recently, two studies have shown that exposure to environmental exposures suspected to be lymphomagens (*i.e.*, trichloroethylene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) pertubated plasma levels of sCD27 and sCD30.[30,31] This may indicate that lymphomagens exert their effect by dysregulation of the immune system as represented by changes in the B-cell activation markers.

A first limitation of our study resides in the fact that we measured blood immune markers at a single time point, which may not reflect accurately the long-term B-cell activation status. However, several studies have provided evidence of favourable within-person temporal stability for panels of cytokines and soluble cytokine receptors.[32–37] In addition, different media for blood samples (citrate in EPIC, Italy and EDTA in NSHDS) might have introduced differences in sCD27 and sCD30 levels between the two cohorts, potentially inducing a bias in unconditional analyses by incomplete correction by cohort status in the model. However, stratified analyses by cohort showed overall consistency of our findings, suggesting a limited impact of this bias in our results. Our meta-analyses were limited by the fact that measured levels of the markers differed by study and therefore different quartiles (including the reference) may not mean the same in each study. Although we tried to solve this problem using an intra-study meta-analysis across all nonreferenced categories this may not have made the studies exactly comparable. In addition, disease definitions and prevalence of specific subtypes differed between studies. The most evident example of this type of heterogeneity related to the definition of BCL, which included in our study multiple myeloma. Analyses excluding MM cases revealed overall similar results (Table 2), and these were subsequently included in our meta-analysis to keep the case definitions similar between studies.

Prospective cohort studies among the general population (including ours) of Western and East Asians countries, especially in subjects with a relatively long duration between blood draw and BCL diagnosis, provide strong and consistent evidence that lymphomagenesis may result from increased levels of B-cell activation, as already reported for HIV/AIDS subjects. The presence of a consistent association of sCD30 with DLBCL and FL and sCD27 and CLL over a number of years (up to 17 years) argues that the association cannot be attributed simply to undiagnosed disease. On the other hand we cannot exclude that due to the long latency time of indolent lymphomas some of the signal might be partially due to early stages of

disease. In conclusion, our findings add to previous analyse to provide evidence that B-cell activation is likely an important phenomena in lymphomagenesis in particular in DLBCL, FL, and CLL.

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