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Circulating sCD27 and sCD30 in pre-diagnostic samples collected fifteen years apart and future non-Hodgkin lymphoma risk

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Abstract

Elevated serum sCD27 and sCD30 from a single banked sample have been associated with future non-Hodgkin lymphoma risk; however, the etiologic relevance of this finding is unclear. To address this question, we conducted a case-control study (235 cases, 235 controls) nested within the CLUE-I and CLUE-II cohorts, which enrolled participants in 1974 and 1989 respectively in Washington County, Maryland. Our study features a subset of 102 cases and 102 controls with two banked pre-diagnostic samples each, collected fifteen years apart. In analyses involving an individual sample per subject, both sCD27 and sCD30 were associated with NHL diagnosed up to 20 years later. In analyses involving repeated samples, cases were significantly more likely than controls to have higher analyte levels in the CLUE-II vs. CLUE-I sample for sCD27 ($P = 0.006$) but not sCD30 ($P = 0.16$). In joint analyses of dichotomized analyte levels in both samples, the strongest NHL association observed for sCD27 was for having below-median levels in CLUE-I and above-median levels in CLUE-II [odds ratio (OR) 3.6, 95% confidence interval (CI) 1.4–9.2 vs. below-median levels in both). In joint analyses for sCD30, the strongest NHL association was observed for having above-median levels in both samples (OR 1.7, 95% CI 0.8–3.7), particularly for cases diagnosed >10 years after the CLUE-II sample (OR 2.4, 95% CI 0.9–6.7). Our findings suggest that sCD27 is a disease marker for NHL and add to the weight of evidence that elevated circulating sCD30 is a marker of increased NHL susceptibility.

Keywords

non-Hodgkin lymphoma; cohort studies; serum; CD27; CD30

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Authorship contributions

Contribution: M.P.P. led the study design and statistical analysis, and prepared the manuscript. Q.L., J.H.B., A.H., P.S., K.V., and N.R. contributed to the study design. Assays were conducted in a laboratory under the direction of A.H.; C.L.C. contributed to the statistical analysis. All authors provided intellectual input into preparation of the manuscript.

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Introduction

Severe immune dysregulation is an established risk factor for non-Hodgkin lymphoma (NHL).[1] Past cohort investigations suggest that subtle immunologic effects may also affect risk, with associations observed for elevated pre-diagnostic circulating levels of soluble CD27 (sCD27) and sCD30, cleaved fragments of the lymphocyte transmembrane proteins CD27 and CD30 measured as markers of immune activation.[2–7] The etiologic relevance of these findings, however, is unclear. Although these associations are compatible with an etiologic role for immunologic mechanisms characterized by elevated sCD27 and sCD30, they may alternatively be symptomatic of a developing lymphoma, or a combination thereof. Past studies have been limited in their ability to address this issue given their use of a single banked sample per participant, which may not adequately capture long-term analyte intra-individual variability, nor changes in levels closer to diagnosis.

The CLUE-I and -II cohorts, with a subset of participants who enrolled in both studies and contributed two blood specimens fifteen years apart, provide a unique resource for advancing our understanding of the temporal relationship between sCD27 and sCD30 levels and NHL risk. We report findings from a nested case-control study within CLUE-I involving 235 case-control matched pairs, 102 of which also had CLUE-II specimens available for analysis.

Methods

Between August and November of 1974, 25,802 adults enrolled in the Campaign Against Cancer and Stroke (referred to as CLUE-I from its slogan, “Give us a Clue to Cancer”) in Washington County, Maryland, USA. A 15mL blood sample and answers to a brief questionnaire were obtained from participants. The blood samples were allowed to clot at room temperature for 30 minutes and then kept at 4° C until the serum was separated, usually 3–4 hours later, then stored at −70° C. A second blood collection survey was conducted between May and October of 1989 in Washington County for the Campaign Against Cancer and Heart Disease (CLUE-II); 9,058 CLUE-I participants also took part in CLUE-II. For CLUE-II, blood was collected in 20ml Vacutainers containing heparin to prevent clotting (Fisher-Scientific, Pittsburgh, PA). The blood was refrigerated at 4° C until centrifugation usually within 6 hours but no longer than 24 hours. Once centrifuged, aliquots of plasma were separated and stored at −70° C. Cancer diagnoses among CLUE-I and CLUE-II cohort participants were identified through linkage with the Washington County Cancer Registry and, since 1992, the Maryland State Cancer Registry.

A schematic diagram outlining the design of this nested case-control study is shown in Supplementary Figure S1. The case series (N=235) included cases of incident NHL (ICD7 200, 202, 204.1) diagnosed between the date of CLUE-I serum collection and September 30, 2013 among participants with available serum who did not have a history of cancer, apart from non-melanoma skin cancer, before diagnosis. Of the 235 selected cases, 102 had also participated in CLUE-II. Controls (N=235) were selected from among individuals who were alive and cancer-free at case diagnosis, with individual matching on race (white, other), sex, date of birth (within 1 year), participation in CLUE (CLUE-I only, both cohorts), date of

blood sample donation (within 15 days), sample storage location (Hagerstown or Baltimore, MD), and number of previous specimen thaws.

sCD27 and sCD30 were measured in duplicate by enzyme-linked immunosorbent assay (Bender Medsystems, Burlingame, USA), with samples from each case-control matched set aliquoted into adjacent wells on the same plate by laboratory personnel blinded to case-control status. Duplicate measurements were averaged to calculate analyte concentrations used in the statistical analysis. Measurements outside the range of the standard curve were excluded (1 sCD27 sample, 19 sCD30 samples). Coefficients of variation (CVs) were calculated using natural log-transformed measurements from blinded quality control replicates (n=52) from pooled sera interspersed within and between batches to assess assay reproducibility. The measurements demonstrated good reproducibility for sCD27 (within-batch CV = 6.2%, total CV = 11.5%) and sCD30 (6.8%, 19.1%).

After excluding three cases diagnosed less than one year after CLUE-I sample collection, 232 cases and 235 controls were included in the analysis of CLUE-I samples. Using conditional and unconditional logistic regression modeling, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) for categorized CLUE-I concentrations of sCD27 and sCD30 as well as continuous analyses of log-transformed analyte concentrations (per log increase). As we observed consistent results across conditional and unconditional models, only findings from unconditional models are reported, in order to avoid loss of both subjects from a matched case-control pair when only one of the two subjects had data available for analysis. Categories of analyte concentrations were defined using control tertiles as cutpoints, with tests of trend performed by modeling the intra-category medians as a continuous parameter. We evaluated the impact of follow-up time from blood collection to case diagnosis on our findings by calculating ORs comparing case subcategories defined by follow-up time (≤20 years, >20 years after CLUE-I sample collection) to all controls using unconditional logistic regression with adjustment for baseline age, sex and race. We additionally calculated ORs relating log-transformed continuous sCD27 and sCD30 with selected histologic subtypes (diffuse large B-cell lymphoma [DLBCL], chronic lymphocytic leukemia/small lymphocytic lymphoma [CLL/SLL], follicular lymphoma [FL], other or not otherwise specified histology [NOS]) using polytomous regression adjusting for the aforementioned covariates.

Additional analyses were performed within the subset of participants with pre-diagnostic measurements from CLUE-I and CLUE-II samples. After excluding 19 cases diagnosed prior to (N=12) or less than one year after (N=7) the CLUE-II sample collection date to avoid reverse causation bias, 83 cases and 102 controls were included in these analyses. We computed Pearson correlation coefficients among controls relating (1) log-transformed concentrations of sCD27 and sCD30 for a given sample and (2) comparisons of measurements across samples for a given analyte. Analyte metrics analyzed included categories of concentration in the CLUE-II sample (≤ vs. > median among controls), the difference in analyte concentration between the CLUE-II and CLUE-I samples (≤0, 0 – above-zero median among controls, > above-zero median among controls), and a joint classification of dichotomized CLUE-I and CLUE-II concentrations (cutpoint: median among controls) for an analyte (i.e., low / low, low / high, high / low, and high / high for

CLUE-I / CLUE-II samples respectively). Conditional and unconditional logistic regression models were fit for analyses of overall NHL, and unconditional logistic regression models were used to calculate ORs for cases diagnosed at different periods of follow-up (<10 years, ≥10 years after CLUE-II sample collection). Polytomous regression was performed to estimate subtype-specific associations. All statistical tests were two-sided.

Results

As summarized in Table 1, the CLUE-I cases and controls were comparable in age at enrollment (median: 49 years), sex (56% female) and race (99% white). The subset enrolled in both CLUE-I and CLUE-II was slightly younger (median age at CLUE-I enrollment: 43 years) than other CLUE-I participants, but otherwise comparable. The median length of follow-up from CLUE-I sample collection to diagnosis was 20.9 years for all cases, while for cases with pre-diagnostic samples from both CLUE-I and CLUE-II, the median follow-up length from the CLUE-II collection date to diagnosis was 10.4 years. The distribution of NHL histologic subtypes was comparable between the two case groups. Among controls enrolled in both CLUE-I and CLUE-II, sCD27 and sCD30 within the same sample were moderately correlated with one another (CLUE-I, r = 0.36; CLUE-II, r = 0.48). Correlations across CLUE-I and CLUE-II samples for sCD27 and sCD30 were 0.60 and 0.26 respectively.

Results from case-control comparisons of sCD27 and sCD30 concentrations in CLUE-I samples are summarized in Table 2. CLUE-I sCD27 was not related to NHL overall, although in analyses restricted to cases diagnosed within 20 years of CLUE-I sample collection, an association was observed (OR 2.8, 95% CI 1.3–6.0 per log increase). Similarly, sCD30 was associated with NHL cases diagnosed within 20 years of CLUE-I sample collection (OR 1.7, 95% CI 1.0–2.8 per log increase), but not for those diagnosed >20 years after enrollment or for cases overall. In analyses of NHL subtypes diagnosed within 20 years of sample collection, associations with sCD27 and sCD30 in CLUE-I samples were stronger for FL (ORs 4.8 and 3.7 per log increase, respectively) than other subtypes, although confidence intervals were wide and tests of OR heterogeneity were not statistically significant (Supplementary Table S1).

Findings from analyses of sCD27 restricted to subjects enrolled in both CLUE-I and CLUE-II are summarized in Table 3. sCD27 in the CLUE-II sample was associated with NHL (high vs. low: OR 2.9, 95% CI 1.5–5.5). In analyses of the difference in levels between cohort samples, cases were significantly more likely than controls to have a higher concentration of sCD27 in the CLUE-II vs. the CLUE-I sample (OR 3.0, 95% CI 1.4–6.4 for difference >0.52 vs. ≤0 U/mL; P_{trend} = 0.006); this was particularly evident for cases diagnosed within 10 years of the CLUE-II sample collection (OR 4.2, 95% CI 1.7–10.7; P_{trend} = 0.002) and for cases of CLL/SLL histology (OR 6.0, 95% CI 1.6–22.1 P_{trend} = 0.006). In a joint analysis of CLUE-I and CLUE-II measurements, the strongest NHL association was observed for having low CLUE-I sCD27 and high CLUE-II sCD27 (“low / high”; OR 3.6, 95% CI 1.4–9.2 vs. low in both samples). Similar findings were observed for cases diagnosed ≤10 years and >10 years after the CLUE-II sample collection. In a joint analysis by subtype, the low / high association was strongest for CLL/SLL (Supplementary Table S2).

As shown in Table 3, CLUE-II sCD30 was also associated with NHL (OR 1.8, 95% CI 1.0–3.5). In comparisons between repeated samples, sCD30 levels were higher in the CLUE-II vs. the CLUE-I sample for the majority of cases and controls; in a comparison across study groups, the proportion of subjects with a positive slope was non-significantly higher among cases (OR 1.7, 95% CI 0.8–3.8 for difference >0.58 vs. ≤0 ng/mL; $P_{trend} = 0.16$). In a joint analysis of sCD30 measurements in CLUE-I and CLUE-II samples, the strongest NHL association was observed for having high sCD30 levels in both samples (OR 1.7, 95% CI 0.8–3.7), particularly for cases diagnosed >10 years after the CLUE-II collection (OR 2.4, 95% CI 0.9–6.7). No clear differences in the joint-analysis findings by subtype were apparent, although the confidence intervals are wide due to small numbers (Supplemental Table S2).

Discussion

Findings from this study, featuring a subset of participants with two banked specimens collected fifteen years apart, provide insight into the temporal nature of NHL risk associations with pre-diagnostic circulating sCD27 and sCD30. In analyses involving measurements in a single pre-diagnostic sample per subject, as was typically performed in past studies, we observed associations between elevated circulating levels of sCD27 and sCD30 and increased risk of NHL diagnosed within 20 years of blood collection, consistent with previous reports.[2–7] However, among participants with two samples, cases were significantly more likely than controls to have higher sCD27 levels in the CLUE-II vs. the CLUE-I sample. For sCD30, the case-control difference in slope was weaker and not statistically significant. In joint analyses, the strongest association for sCD27 was observed for subjects with a low CLUE-I concentration and high CLUE-II concentration, while for sCD30 the strongest association was observed for subjects with high concentrations in both samples. We interpret our findings for sCD27 to be reflective largely of a disease effect, and our sCD30 results to be more compatible with an underlying etiologically relevant effect.

Our results are consistent with findings from two recent studies with similarly long follow-up. In a study within the Alpha Tocopherol Beta-Carotene Cancer Prevention Study, the findings for circulating sCD27 in analyses stratified across different periods of follow-up were consistent with reverse causation; the association with NHL was strongest for cases diagnosed less than eight years after blood collection, weaker for cases diagnosed 8 to 14 years post-collection, and null for cases diagnosed 15 to 23 years later.[7] In contrast, associations with sCD30 were consistent across the three periods of follow-up. A recent study by Späth et al. nested within the Northern Swedish Health and Disease Study cohort is, to our knowledge, the only other besides our own to use repeated samples, with samples collected an average of eight years apart.[8] In comparisons between repeated samples, a rise in serum levels of sCD27 and sCD30 was observed for cases, particularly those diagnosed within 3 years of the second sample collection, consistent with a disease-induced effect. However, the investigators also found elevated levels of both markers to be significantly associated with NHL risk for cases diagnosed 15–25 years after blood collection, compatible with an underlying mechanism of etiologic relevance.

In our study, the rise in sCD27 closer to case diagnosis was particularly strong for CLL/SLL. Similarly, CLL had the strongest sCD27 trajectory among NHL subtypes investigated by Späth et al., and the strongest association with sCD27 in a recent meta-analysis.[9] It is plausible for elevated sCD27 to have been induced by this NHL subtype, as serum concentrations of this analyte has been reported in clinical studies to be correlated with CLL stage and tumor load.[10, 11] Our findings and those of Späth et al. raise the possibility that sCD27 measurements may have clinical value for CLL/SLL surveillance among patients with monoclonal B-cell lymphocytosis, an asymptomatic condition preceding this malignancy.[12]

Our sCD30 results are consistent with an etiologic relationship, given the lack of clear evidence of an increasing trajectory among cases and an association with risk for consistently elevated levels, particularly for cases diagnosed over ten years after CLUE-II sample collection. sCD30 has been proposed to be a potential marker of B-cell activation,[13] as CD30 is preferentially expressed by activated type-2 T cells, which produce cytokines enhancing B-cell activation.[14, 15] A state of consistently elevated B-cell activation increases the potential for unrepaired genetic errors in B lymphocytes undergoing clonal proliferation, somatic recombination and class switching, potentially leading to NHL.

Our study, although not the first to use repeated samples to investigate the relationship between these markers and NHL, complements the findings of Späth et al. given its longer time between the collection of samples (fifteen years vs. an average of eight years), which permitted us to capture the dynamics in analyte levels across a long and uniform span of time. Additionally, our study included a longer period of follow-up after the later banked sample (mean 10.8 years vs. 5.5 years), which enabled us to investigate associations for cases diagnosed over ten years after the second blood collection, where disease-induced effects are less likely to be present. A limitation of our study is the comparatively small number of participants with repeated samples, which led to wide confidence limits around our OR estimates, sometimes including the null. Further, the systematic difference in sample type between CLUE-I and CLUE-II (serum and plasma, respectively) may have contributed to extraneous variability to our comparisons across samples, although this would be common to cases and controls alike.

In conclusion, our findings from this nested case-control study of circulating sCD27 and sCD30 NHL, featuring a subset of participants with two banked specimens collected nearly fifteen years apart, suggest that reported case-control associations with sCD27 may reflect occult disease, CLL/SLL in particular. In addition, our observed association with NHL for consistently elevated sCD30 is consistent with the hypothesis that elevated circulating levels of this analyte is a marker of increased susceptibility. Additional studies with repeated pre-diagnostic samples are needed to further investigate the dynamics of these and other circulating immune markers in relation to NHL, both from the standpoint of the insight they can provide into disease etiology as well as their potential clinical value, such as evaluating sCD27 trajectory as a predictor of progression from MBL to CLL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Impact:

The blood markers sCD27 and sCD30 have been reported to be elevated in blood collected several years prior to the diagnosis of non-Hodgkin lymphoma. However, the use of a single pre-diagnostic sample per subject in past studies has limited inferences regarding an etiologic or prodromal effect. Findings from this novel study, which included pre-diagnostic samples collected fifteen years apart, suggest that sCD27 is an early-disease marker, while sCD30 may be a marker of increased susceptibility.

Table 1.

Selected characteristics of non-Hodgkin lymphoma cases and controls selected from the CLUE-I and CLUE-II cohorts

	All Participants		Subset of Participants in Both CLUE-I and CLUE-II	
	Cases	**Controls**	**Cases**	**Controls**
Participants	232	235	83*	102
Median years of age at CLUE-I enrollment (min, max)	49 (14, 78)	49 (14, 79)	43 (14, 68)	43 (14, 67)
Percent female	55.6	55.8	59.0	56.7
Percent white	99.1	98.7	98.8	98.0
Median years from sample collection to case diagnosis (min, max)	20.9 (2.1, 39.0)		10.4 (1.0, 24.3)†	
Median years between CLUE-I and CLUE-II sample collection			14.8 (14.5, 15.1)	14.8 (14.5, 15.1)
Case histology				
DLBCL	85		35	
CLL / SLL	65		22	
FL	33		11	
Other / NOS	49		15	

Abbreviations: SD, standard deviation; DLBCL, diffuse large B-cell; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; FL, follicular lymphoma; NOS, not otherwise specified.

*Excludes 19 cases diagnosed before or less than one year after CLUE-II sample collection date.

†Years from CLUE-II sample.

Table 2.

NHL associations with CLUE-I serum sCD27 and sCD30 levels, overall and by follow-up length from CLUE-I sample to case diagnosis

					Years from CLUE-I sample collection to diagnosis			
					<20		21 – 39	
Analyte	**Metric**	**N_{cont}**	**N_{Ca}**	**OR* (95% CI)**	**N_{Ca}**	**OR (95% CI)**	**N_{Ca}**	**OR (95% CI)**
sCD27, U/mL	≤45.6	79	66	1.0	26	1.0	40	1.0
	45.7 – 58.1	78	87	1.3 (0.9–2.1)	39	1.4 (0.8–2.6)	48	1.4 (0.8–2.3)
	>58.1	78	77	1.2 (0.8–1.9)	51	1.8 (1.0–3.2)	26	0.8 (0.4–1.4)
				P_{trend} = 0.49		P_{trend} = 0.06		P_{trend} = 0.49
	per log increase			1.6 (0.8–3.0)		2.8 (1.3–6.0)		0.7 (0.3–1.7)
sCD30, ng/mL	≤23.82	78	69	1.0	28	1.0	41	1.0
	23.83 – 34.00	78	81	1.2 (0.8–1.9)	35	1.3 (0.7–2.4)	46	1.1 (0.6–1.9)
	>34.00	79	74	1.1 (0.7–1.7)	49	1.7 (1.0–3.0)	25	0.6 (0.3–1.2)
				P_{trend} = 0.81		P_{trend} = 0.07		P_{trend} = 0.13
	per log increase			1.2 (0.8–1.9)		1.7 (1.0–2.8)		0.8 (0.5–1.4)

Abbreviations: Cont, controls; Ca, cases; OR, odds ratio; CI, confidence interval.

*OR calculated using unconditional logistic regression adjusting for age at CLUE-I enrollment, sex and race.

Table 3.

Different measures of NHL association with serum sCD27 and sCD30 levels among subjects with pre-diagnostic samples from both CLUE-I and CLUE-II (overall and by follow-up length from CLUE-II sample to diagnosis)

					Years from CLUE-II sample collection to diagnosis			
					≤10		11 – 24	
Analyte	**Metric**	**N_{cont}**	**N_{Ca}**	**OR* (95% CI)**	**N_{Ca}**	**OR (95% CI)**	**N_{Ca}**	**OR (95% CI)**
sCD27, U/mL	Concentration in CLUE-II sample							
	≤47.3	51	24	1.0	11	1.0	13	1.0
	>47.3	50	59	2.9 (1.5–5.5)	37	3.3 (1.5–7.5)	22	2.5 (1.1–5.9)
	Difference between samples (CLUE-II - CLUE-I)							
	≤0	46	25	1.0	10	1.0	15	1.0
	>0–0.52	28	23	1.7 (0.8–3.7)	13	2.1 (0.8–5.5)	10	1.5 (0.6–4.0)
	>0.52	27	34	3.0 (1.4–6.4)	25	4.2 (1.7–10.7)	9	1.7 (0.6–4.9)
				P_{trend} = 0.006		P_{trend} = 0.002		P_{trend} = 0.31
	Joint classification across samples (CLUE-I/CLUE-II)†							
	Low / Low	37	18	1.0	8	1.0	10	1.0
	Low / High	13	20	3.6 (1.4–9.2)	13	4.6 (1.5–13.8)	7	3.2 (0.9–11.5)
	High / Low	14	5	0.7 (0.2–2.3)	3	1.0 (0.2–4.4)	2	0.5 (0.1–2.7)
	High / High	37	39	2.4 (1.1–5.1)	24	2.9 (1.1–7.5)	15	2.0 (0.7–5.3)
sCD30, ng/mL	Concentrationin CLUE-II sample							
	≤30.70	50	31	1.0	16	1.0	15	1.0
	>30.70	49	49	1.8 (1.0–3.5)	29	1.7 (0.8–3.7)	20	1.8 (0.8–4.1)
	Difference between samples (CLUE-II - CLUE-I)							
	≤0	30	19	1.0	8	1.0	11	1.0
	>0–0.58	33	24	1.2 (0.5–2.7)	13	1.5 (0.5–4.1)	11	1.1 (0.4–3.0)
	>0.58	34	33	1.7 (0.8–3.8)	20	2.1 (0.8–5.7)	13	1.3 (0.5–3.7)
				P_{trend} = 0.16		P_{trend} = 0.13		P_{trend} = 0.56
	Joint classification across samples (CLUE-I/CLUE-II)‡							
	Low / Low	30	21	1.0	12	1.0	9	1.0
	Low / High	18	15	1.3 (0.5–3.3)	11	1.4 (0.5–4.1)	4	0.9 (0.2–3.4)
	High / Low	19	9	0.6 (0.2–1.7)	3	0.4 (0.1–1.6)	6	1.0 (0.3–3.4)
	High / High	30	31	1.7 (0.8–3.7)	15	1.2 (0.4–3.1)	16	2.4 (0.9–6.7)

Abbreviations: Cont, controls; Ca, cases; OR, odds ratio; CI, confidence interval.

*OR calculated using unconditional logistic regression adjusting for age at CLUE-I enrollment, sex and race.

†sCD27 category cutpoints: CLUE-I, ≤ vs. > 48.1 U/mL; CLUE-II, ≤ vs. > 47.3 U/mL

‡sCD30 category cutpoints: CLUE-I, ≤ vs. > 27.79 ng/mL; CLUE-II, ≤ vs. > 30.70 ng/mL