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Soluble levels of CD27 and CD30 are associated with risk of non-Hodgkin lymphoma in three Chinese prospective cohorts

Bryan A. Bassig¹, Xiao-Ou Shu², Woon-Puay Koh³, Yu-Tang Gao⁴, Mark P. Purdue⁵, Lesley M. Butler^{6,7}, Jennifer Adams-Haduch⁶, Yong-Bing Xiang⁸, Troy J. Kemp⁹, Renwei Wang⁶, Ligia A. Pinto⁹, Tongzhang Zheng¹⁰, Bu-Tian Ji¹, H. Dean Hosgood¹¹, Wei Hu¹, Gong Yang², Heping Zhang¹⁰, Wong-Ho Chow¹², Christopher Kim¹, Wei Jie Seow¹, Wei Zheng^{2,*}, Jian-Min Yuan^{6,7,*}, Qing Lan^{1,*}, and Nathaniel Rothman^{1,*}

¹ Division of Cancer Epidemiology and Genetics, NCI, NIH, DHHS, Rockville, MD, USA

² Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN, USA

³ Duke-NUS Graduate Medical School Singapore, Singapore

⁴ Department of Epidemiology, Shanghai Cancer Institute, Shanghai Jiaotong University, Shanghai, China

⁵ Ontario Institute for Cancer Research, Toronto, Ontario, Canada

⁶ Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

⁷ Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

⁸ Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

⁹ HPV Immunology Laboratory, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD USA

¹⁰ Yale University School of Public Health, New Haven, CT, USA

¹¹ Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York, NY, USA

¹² Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Abstract

Correspondence: Bryan Bassig, Ph.D., M.P.H. Occupational & Environmental Epidemiology Branch Division of Cancer Epidemiology and Genetics National Cancer Institute, NIH 9609 Medical Center Drive, MSC 9776 Rm. 6E618 Bethesda, Maryland 20892 Telephone: 240-276-7441 bassigb@mail.nih.gov.

*These authors co-supervised this work

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Prospective studies conducted in Western populations have suggested that alterations in soluble CD27 (sCD27) and soluble CD30 (sCD30), two markers indicative of B-cell activation, are associated with risk of non-Hodgkin lymphoma (NHL). Given that the characteristics of NHL in East Asia differ from the West, and mechanistic commonalities between these populations with respect to the role of intermediate endpoint biomarkers in lymphomagenesis have not been explored, we conducted a pooled nested case-control study from three prospective studies of Chinese men and women including 218 NHL cases and 218 individually matched controls. Compared to the lowest quartile, ORs (95% CIs) for the 2nd, 3rd, and 4th quartiles of sCD27 were 1.60 (0.83-3.09), 1.94 (0.98-3.83), and 4.45 (2.25-8.81), respectively ($p_{\text{trend}} = 0.000005$). The corresponding ORs for sCD30 were 1.74 (0.85-3.58), 1.86 (0.94-3.67), and 5.15 (2.62-10.12) ($p_{\text{trend}} = 0.0000002$). These associations remained statistically significant in individuals diagnosed with NHL 10 or more years after blood draw. Notably, the magnitude of the associations with NHL risk was very similar to those in Western populations in previous studies. These findings of the similar association between sCD27 or sCD30 and NHL risk across different populations support an important underlying mechanism of B-cell activation in lymphomagenesis.

Keywords

CD27; CD30; immune markers; non-Hodgkin lymphoma; East Asians; prospective study

INTRODUCTION

Epidemiological studies conducted in Western populations have suggested a strong relationship between immune suppression, underlying immune dysfunction, and subsequent risk of non-Hodgkin lymphoma (NHL), as an elevated risk of NHL has been observed for multiple viral infections, including HIV, in post-transplant patients, and for specific autoimmune diseases.^{1,2} Moreover, there is increasing evidence from prospective studies conducted in Western populations that more subtle immune disturbances, such as alterations in circulating cytokines and other immune markers, also influence NHL susceptibility, and that chronic B-cell activation may precede lymphomagenesis.^{3,5} These markers that have been implicated in lymphomagenesis include levels of soluble CD27 (sCD27) and soluble CD30 (sCD30), higher levels of which were initially demonstrated to be associated with increased risk of NHL in HIV-infected individuals,^{6,7} as well as in healthy Western populations.^{4,8,11} The elevations in these markers have been seen in blood samples collected from individuals more than 10 years before they were diagnosed with NHL.^{10,11}

CD27 and CD30 are both members of the tumor necrosis factor superfamily that are expressed on specific subsets of lymphocytic cells, including memory B lymphocytes, and these markers have broad immunoregulatory activities upon activation, including promotion of lymphocyte survival and proliferation and regulation of Th1/Th2 immune responses.^{12,13} sCD27 and sCD30 are cleaved from activated lymphocytes that express CD27 and CD30, and are thus useful indicators of immune activation that have been used clinically to monitor immune activity and progression of several conditions including HIV and autoimmune disease.^{13,15}

Several recent prospective epidemiologic studies have evaluated associations between sCD27 and/or sCD30 and risk of NHL in healthy populations, all of which have been in Western populations and the majority of whom were of European descent.^{4,8-11} In each of these studies, elevated levels of sCD27 and sCD30 were strongly associated with increased risk of NHL, with ORs ranging from 2.8-5.5 in overall analyses comparing the highest quartile or tertile of sCD27 or sCD30 to the lowest (see Supplemental Table 1). However, there are currently no data evaluating whether these biomarkers are associated with NHL risk in East Asian populations.

Evaluation of risk factors for NHL in the East Asian population, particularly those that have been strongly associated with lymphomagenesis in Western populations, is of particular interest given that the descriptive characteristics of NHL in terms of histological subtype distributions and incidence rates are quite different from those in Western countries, and there is currently limited understanding of the factors responsible for these differences.^{16,17} For instance, higher incidence rates and subtype distributions of some NHL subtypes derived from T- or NK/T cells, particularly extranodal NK/T cell lymphoma, have been reported in East Asia compared to the West, whereas lower proportions and rates of some B-cell subtypes, including follicular lymphoma (FL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), have been observed in East Asia.^{16,18} The overall incidence rates of NHL are about three times lower in Shanghai and about two times lower in Singapore compared to those in the United States.¹⁷ Further, given that there is evidence for genetic diversity in key immunoregulatory genes between Chinese individuals and those of European descent,¹⁹ and possible geographic differences in exposure to viral and/or environmental factors that may contribute to immune stimulation, the extent to which these markers, and subclinical immune alterations more generally, are similarly associated with subsequent NHL risk in East Asians is currently unknown. Thus, to evaluate whether levels of these markers are associated with NHL risk in a population of East Asian individuals, we pooled data from three population-based prospective cohorts of Chinese men and women from Shanghai and Singapore, including a total of 218 NHL cases and 218 controls.

MATERIALS AND METHODS

Study Population

Cases of NHL and individually matched controls for this pooled study were identified from three prospective cohorts in Shanghai and Singapore, including the Shanghai Women's Health Study (SWHS), the Shanghai Cohort Study (SCS), and the Singapore Chinese Health Study (SCHS). The design and populations for each of these cohorts have been described in detail.^{20,22} Briefly, the SWHS is a prospective cohort of 74,942 women living in urban Shanghai who were enrolled between the ages of 40 and 70 with subject enrollment occurring between December 1996 and May 2000.²⁰ Eligible women for the study were identified from a roster of all women from the resident offices in the study communities and were approached by a trained interviewer and a local community health worker. Enrolled women completed a questionnaire that included an assessment of demographic characteristics, personal lifestyle habits, medical history, and a residential history. Biological samples were obtained from women at the time of the baseline interview. The SCS includes

18,244 men enrolled between 1986 and 1989 who were between 45 and 64 years old at enrollment and resided in one of four geographically defined communities in Shanghai.²¹ Biological samples were collected at the time of the in-person interview, which was conducted by trained nurses. Finally, the SCHS is a population-based prospective cohort including 63,257 Chinese men and women who were aged 45-74 years at the time of enrollment in 1993-1998.²² Subjects for this study were recruited from public housing estates in Singapore, which about 85% of the population in Singapore resided in during the years of enrollment. A structured questionnaire was completed through in-person interviews at recruitment. Biologic specimens were collected for cohort participants who consented to donate biospecimens for research following the completion of the first follow-up interview during 2000-2004. All studies were approved by the appropriate Institutional Review Boards.

Case ascertainment for the cohorts was conducted primarily through annual record linkage to the Shanghai Cancer Registry for the SWHS and SCS and to the Singapore Cancer Registry for the SCHS. For the SCS, annual in-person follow-up interviews to surviving cohort members supplemented the identification of new incident cancer cases, whose diagnosis was formed with the review of and abstraction of information from patients' medical records. For this pooled study, all NHL cases (ICD-9 codes 200, 202, and 204 for the SWHS and SCS, and ICDO-2 codes 9590-9595, 9670-9677, 9680-9688, 9690-9698, 9700-9717, and 9820-9828 for the SCHS) with adequate volumes of plasma or serum available for analyses were identified and individually matched in a 1:1 ratio to controls. To be eligible, all controls were alive and free of cancer at the time of the NHL diagnosis of the index case. For each cohort, we had already developed the specific matching criteria for control selection. For the SCS, the matching criteria were age at baseline interview (± 2 years), gender, date of blood collection (± 1 month), and neighborhood of residence at the time of recruitment. For the SCHS, the matching criteria included age at baseline interview (± 3 years) and date of baseline interview (± 2 years), date of biospecimen collection (± 6 months), gender, and dialect group (Cantonese, Hokkien). For the SWHS, the matching criteria included age at baseline interview (± 5 years) and date of blood collection (± 6 months). All controls were matched to cases from the same study. A total of 218 cases and 218 matched controls were identified and included in the analyses, including 99 case-control pairs from the SWHS, 71 case-control pairs from the SCS, and 48 case-control pairs from the SCHS.

Laboratory Analysis

Plasma or serum levels of sCD27 and sCD30, depending on the type of blood sample available from each cohort, were measured in duplicate using enzyme-linked immunosorbent assay (Bender Medsystems) at the HPV Immunology Laboratory at the Frederick National Laboratory for Cancer Research (SAIC, Frederick). Samples from each of the three cohorts were tested at the same time and the average of two duplicate measurements was used for analyses. The laboratory investigators involved in the measurement of sCD27 and sCD30 were blind to the case-control status of all of the samples included in the study. Cases and their matched controls were analyzed consecutively in the same batch and duplicate and replicate quality control samples were interspersed throughout

the batches to evaluate intra- and inter-batch reproducibility in the measurements. The overall intra- and inter-coefficients of variation (CV) for the sCD27 assay were 4.9% and 14.6%, respectively, and for the sCD30 assay were 4.3% and 13.6%, respectively.

Statistical Analysis

Differences in median concentrations of sCD27 and sCD30 between NHL cases and their matched controls were evaluated using the Wilcoxon signed-rank test for matched pairs. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for analyte levels and overall NHL risk using the lowest quartile as the reference group, based on the distribution in all study controls. Models were adjusted for age as a continuous variable, in order to account for potential residual confounding, as well as for ever smoking. Tests for linear trend were conducted by modeling the median values of the analyte quartiles as a continuous variable. Stratified analyses based on the time period between blood collection and NHL diagnosis were conducted based on the median follow-up period (7 years) and within three time period strata based on tertiles of follow-up time in the cases (0– 4.5 years, >4.5– <10 years, and ≥ 10 years), using polytomous logistic regression and all controls as the reference group, adjusted for age, gender, study, year of blood collection, and smoking status (never, ever). For these stratified analyses, tertile distributions of the markers were used based on the distributions in all controls. Comparisons of the effects across these case categories were conducted using Wald tests within the polytomous logistic regression models. Further, study-specific associations between both markers and NHL risk were evaluated using conditional logistic regression, using tertiles based on the distributions of the study-specific controls. Most NHL cases were classified as other lymphomas (e.g. ICD-9 202.8) in these cohorts, which precluded detailed analyses according to NHL subtype, and therefore the primary results presented are for overall NHL. However, we conducted exploratory analyses in this population for both markers in association with risk of lymphoid leukemia (ICD-9, 204) separately, based on 27 cases, using unconditional logistic regression and compared to all controls as the reference. All statistical tests were 2-sided with $p < 0.05$ considered statistically significant. Statistical Analysis Software (SAS v. 9.3, Cary, NC) was used to conduct all analyses.

RESULTS

Selected characteristics of the NHL cases and matched controls included in this study are shown in Table 1. The pooled study population was evenly distributed with respect to age at enrollment (mean = 57 years) and gender (52% female). Over 90% of subjects in the SWHS were non- smokers whereas ever smokers in both cases and controls were more common in the SCS and SCHS (Table 1).

The median length of time between blood collection and NHL diagnosis for cases in each of the three studies was 7.6 years in the SWHS, 13.6 years in the SCS, 2.8 years in the SCHS, and 7.2 years overall. The mean age of the cases at diagnosis of NHL was 66 years. Median concentrations of these analytes in the pooled study population were higher in NHL cases compared to matched controls for both sCD27 ($p < 0.0001$) and sCD30 ($p < 0.0001$; Table 2 and Supplemental Table 2).

Conditional logistic regression analyses by quartile based on the analyte distributions in all study controls are shown in Table 2. Higher levels of both sCD27 and sCD30 were associated with significantly increased risk of NHL (p_{trend} both < 0.0001). Compared to the lowest quartile, ORs (95% CIs) for the 2nd, 3rd, and 4th quartile of sCD27 were 1.60 (0.83-3.09), 1.94 (0.98-3.83), and 4.45 (2.25-8.81), respectively. The corresponding ORs for sCD30 were 1.74 (0.85-3.58), 1.86 (0.94-3.67), and 5.15 (2.62-10.12). Significant elevations in the highest quartile for sCD27 (OR = 3.76, 95% CI = 1.80-7.85; $p_{trend} = 0.0003$) and sCD30 (OR = 5.13, 95% CI = 2.46-10.69; $p_{trend} = 0.00001$) were also observed in sensitivity analyses that excluded cases with < 2 years between blood collection and diagnosis, in order to account for the potential influence of undiagnosed disease on levels of these markers. There was no statistical evidence of heterogeneity in these associations by study for either sCD27 ($p = 0.45$) or sCD30 ($p = 0.29$) (Supplemental Table 3).

There was a moderate degree of correlation between continuous levels of sCD27 and sCD30 in both controls ($r_{sp} = 0.23$, $p = < 0.01$) and cases ($r_{sp} = 0.45$, $p = < 0.01$). The highest quartile for each of these markers remained significantly elevated for risk of NHL when both were included in the model simultaneously. The mutually adjusted ORs (95% CI) for the 4th vs. 1st quartile were 2.23 (1.02-4.89) for sCD27 and 3.67 (1.70-7.93) for sCD30.

Exploratory analyses for risk of lymphoid leukemia ($n = 27$ cases) showed a statistically significantly increased risk for the highest tertile of sCD27 (OR = 7.01, 95% CI 1.99-24.66) and sCD30 (OR = 5.38, 95% CI = 1.75-16.59) relative to the lowest tertile (data not shown). When cases of lymphoid leukemia and their matched controls were removed from the analysis, the associations between sCD27 or sCD30 and risk of other NHL ($n = 191$ cases) remained statistically significant. Compared with the lowest quartile, the ORs (95% CIs) for the 2nd, 3rd, and 4th quartiles of sCD27 were 1.30 (0.66-2.55), 1.89 (0.92-3.90), and 3.84 (1.87-7.87), respectively ($p_{trend} = 0.00006$). The corresponding ORs for sCD30 were 1.79 (0.83-3.86), 1.83 (0.89-3.79) and 4.69 (2.28-9.63) ($p_{trend} = 0.000006$).

Associations stratified by the time period between date of blood collection and NHL case diagnosis and using all controls as the reference are shown in Tables 3-4. Associations for both markers were stronger in individuals with < 7 years between blood collection and diagnosis, but the effects retained statistical significance in those subjects with > 7 years of follow-up. The ORs (95% CIs) for the 2nd and 3rd tertiles relative to the 1st tertile for sCD27 were 1.73 (0.89-3.38) and 2.65 (1.36-5.19), respectively; and the corresponding results for sCD30 were 1.32 (0.70-2.49) and 2.69 (1.47-4.94). We further stratified the associations by the tertile distributions of follow-up time among cases, and observed that both markers were significantly associated with NHL risk for the longest period of time between blood collection and diagnosis (i.e. ≥ 10 years). Specifically, ORs (95% CIs) for the 2nd and 3rd tertiles relative to the 1st tertile of sCD27 were 1.99 (0.89-4.47) and 2.91 (1.28-6.61), respectively, among cases that were diagnosed more than 10 years after blood draw ($p_{trend} = 0.01$). The corresponding ORs and 95% CIs for sCD30 were 2.12 (0.94-4.75) and 3.83 (1.72-8.56) ($p_{trend} = 0.001$). There was no significant difference in the biomarker-NHL risk associations across these three time periods when comparing the risk associated with the highest tertile ($p = 0.80$ for sCD27 and $p = 0.49$ for sCD30).

DISCUSSION

Epidemiologic evidence based primarily on observations in individuals of European descent has indicated that sCD27 and sCD30 are important biomarkers that are predictive of future risk of NHL, with associations persisting in individuals with greater than 10 years between blood collection and diagnosis in some studies.^{10,11} In this case-control study nested in three population-based prospective cohorts of Chinese men and women living in Shanghai and Singapore, we found that these markers are similarly associated with NHL risk. These associations persisted in subjects with the longest time period between blood collection and NHL diagnosis (i.e. 10 years), an observation that is consistent with previous studies in Western populations and one that potentially supports an important underlying mechanism of B-cell activation in NHL development across populations.

Our study in Eastern Asians is preceded by five prospective studies that have evaluated one or both of these markers in relation to NHL risk in primarily individuals of European descent living in the United States or Europe. The largest of these studies, which included 491 NHL cases and 491 controls enrolled in the Women's Health Initiative Observational Study, reported a stronger association with risk of NHL associated with elevated sCD27 (OR = 5.5) than sCD30 (OR = 2.8) when comparing the highest quartile to the lowest.¹⁰ A somewhat stronger association, in terms of the magnitude of the OR, was also reported for sCD27 among 297 cases and 297 controls from the prospective Prostate Lung Colorectal and Ovarian (PLCO) Cancer Screening Trial,⁴ although both markers remained strongly associated with NHL by as much as 10 years between blood collection and diagnosis.⁹ Most recently, associations with NHL for both markers were replicated among 272 NHL cases and 325 controls from a prospective cohort of male smokers from Finland with a long period of follow-up (i.e. up to 23 years between blood collection and diagnosis).¹¹ Notably, significantly elevated associations with NHL were observed in that study for sCD27 and sCD30 in individuals with up to 15 and 23 years between blood collection and diagnosis, respectively.

Our analyses among Chinese individuals suggested relatively similar magnitude of associations between levels of sCD27 or sCD30 and NHL risk. Further, the strength of the associations for sCD27 and particularly sCD30 in subjects with the longest follow-up time from blood draw to NHL diagnosis was relatively strong and the effect estimates across time periods for the highest tertile of both markers were not significantly different. These two markers were moderately correlated with each other in our study population. The mutual adjustment for each other attenuated the sCD27-NHL risk association more greatly than the sCD30-NHL risk association, although both associations remained statistically significant. Thus, the comparable magnitude of these associations in Western populations and East Asians, especially in subjects with a relatively long duration between blood draw and NHL diagnosis, provides strong support that these markers, which are derived from B-cell activation, may play an important role in the development of NHL in humans.

The mechanism by which elevated levels of sCD27 and sCD30 result in the development of NHL has not been clearly understood. sCD27 has been shown to be a functional protein that acts as an agonist in T-cell activation, as well as enhancing immunoglobulin production and

activating B-cells.^{23,24} Similarly, CD30 is expressed on activated B- and T-lymphocytes and has been demonstrated to promote T-helper 2 (Th2) cells and in addition has been shown to exert immunoregulatory functions partially through mediating Th1 and Th2 cytokine production.¹² Associations with these markers therefore provide evidence for a role of subclinical immune alterations and lymphocyte activation in the etiology of NHL. Given the relatively strong and consistent associations between higher levels of these markers and NHL risk across multiple populations, it would be of interest to evaluate the relationship between suspected lifestyle and environmental lymphomagens and their effects on levels of sCD27 and sCD30, which could provide insight into the biologic plausibility or mechanistic basis of these exposure-NHL associations.

There are several strengths and novel aspects to our study. First, the pooled nature of the study allowed for investigation of these markers for the first time in three prospective studies in the East Asian population with pre-diagnostic specimens and relatively long follow-up, which enabled an assessment of these markers up to > 10 years between blood collection and diagnosis in this population. Notably, ~45% of subjects in this longest time period strata were diagnosed > 15 years after blood collection. Second, given that NHL is a rare disease in the East Asian population, this pooling effort allowed for a relatively large study that was well-powered to detect associations with these markers given the large magnitude of the associations observed in previous studies. A further strength of our study is the measurement of samples from all studies at the same time in the same lab. Cases and their matched controls were measured next to each other on the assay plates and good intra-assay reproducibility was demonstrated on the basis of the calculated CVs.

Given the strong etiologic heterogeneity that has been observed for NHL with respect to several risk factors,²⁵ a limitation of our study is the inability to comprehensively evaluate subtype-specific associations in this population given the size of the study and the lack of detailed histology data for the cases. Some previous studies have suggested associations with these markers for FL and CLL/SLL,^{9,10} while the recent report from the Finnish male cohort reported a significant association between higher levels of sCD30 and risk of diffuse large B-cell lymphoma (DLBCL) that remained elevated in cases with 8-15 years of follow-up after blood collection.¹¹ To explore potential subtype-specific associations in our study, we evaluated the association between these markers and risk of lymphoid leukemia (n=27, 16 CLL/SLL) and NHL excluding lymphoid leukemia cases. Despite the small case numbers, a significantly increased risk of lymphoid leukemia with higher levels of both markers suggest a role of sCD27 and sCD30 in the development of lymphoid leukemia in the Chinese population. Furthermore, observed significant associations between sCD27 or sCD30 and risk of NHL other than lymphoid leukemia indicate that these markers may also be associated with the development of other subtypes of NHL in this population. Given that DLBCL is the most common subtype and accounts for 30%-45% of non-CLL/SLL cases in the Chinese population,^{16,26} it is possible that the associations observed in the present study are most likely driven by the DLBCL subtype. Larger studies in East Asians with detailed histology on NHL cases will be warranted to clarify the associations between these markers and risk of specific subtypes of NHL, and to evaluate if such associations persist after a long duration of follow-up after blood collection prior to the diagnosis of NHL. Identification and replication of strong NHL subtype-specific associations, particularly those that are

suggestive of an etiologic rather than a disease effect, could potentially lead to the translational application of these markers for risk prediction in the future, perhaps in combination with other established risk factors such as findings from recent genome-wide association studies of specific NHL subtypes.

In conclusion, we found that circulating levels of sCD27 and sCD30 measured before NHL diagnosis are positively associated with risk of NHL in the Chinese population and that the associations may precede lymphomagenesis by as much as >10 years before diagnosis. These findings are consistent with those of previous prospective studies in Western populations. The consistent and similar results of the present study along with previous studies implicate an important role of B-cell activation in lymphomagenesis in humans. Future studies are warranted to evaluate the effect of these markers on the risk of specific subtypes of NHL in the East Asian population who have shown different distributions of NHL subtypes from those of Western populations, as well as to delineate the underlying lymphomagenic process that these markers represent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Impact

Soluble levels of CD27 and CD30, two immune markers that are reflective of B-cell activation, are demonstrated for the first time to be associated with future risk of non-Hodgkin lymphoma (NHL) in Chinese men and women. These data provide new insight into lymphomagenesis in Asians and are consistent with findings in Western populations, indicating common mechanistic characteristics for NHL in diverse populations with different patterns of NHL incidence and subtype distributions.

Selected characteristics of cases and controls from a pooled nested case-control study of subjects from the Shanghai Women's Health Study (SWHS), Shanghai Cohort Study (SCS), and the Singapore Chinese Health Study (SCHS)

Table 1

<u>Characteristic</u>	<u>Overall</u>		<u>SWHS</u>		<u>SCS</u>		<u>SCHS</u>	
	<u>Cases</u> (n = 218) <u>n, %</u>	<u>Controls</u> (n = 218) <u>n, %</u>	<u>Cases</u> (n = 99) <u>n, %</u>	<u>Controls</u> (n = 99) <u>n, %</u>	<u>Cases</u> (n = 71) <u>n, %</u>	<u>Controls</u> (n = 71) <u>n, %</u>	<u>Cases</u> (n = 48) <u>n, %</u>	<u>Controls</u> (n = 48) <u>n, %</u>
<u>Age at enrollment</u>								
40-44	12 (6)	14 (6)	11 (11)	11 (11)	1 (1)	2 (3)	0 (0)	1 (2)
45-49	29 (13)	26 (12)	10 (10)	11 (11)	10 (14)	7 (10)	9 (19)	8 (17)
50-54	31 (14)	34 (16)	15 (15)	12 (12)	12 (17)	16 (23)	4 (8)	6 (13)
55-59	48 (22)	47 (22)	14 (14)	15 (15)	22 (31)	20 (28)	12 (25)	12 (25)
60-64	52 (24)	58 (27)	16 (16)	21 (21)	22 (31)	25 (35)	14 (29)	12 (25)
65-69	40 (18)	35 (16)	31 (31)	29 (29)	4 (6)	1 (1)	5 (10)	5 (10)
70	6 (3)	4 (2)	2 (2)	0 (0)	0 (0)	0 (0)	4 (8)	4 (8)
<u>Gender</u>								
Male	104 (48)	104 (48)	0 (0)	0 (0)	71 (100)	71 (100)	33 (69)	33 (69)
Female	114 (52)	114 (52)	99 (100)	99 (100)	0 (0)	0 (0)	15 (31)	15 (31)
<u>Ever Smoking</u>								
Yes	65 (30)	58 (27)	8 (8)	8 (8)	34 (48)	33 (46)	23 (48)	17 (35)
No	153 (70)	160 (73)	91 (92)	91 (92)	37 (52)	38 (54)	25 (52)	31 (65)

Table 2

Associations between sCD27, sCD30, and risk of NHL in a pooled analysis of three prospective cohorts of Chinese men and women in Shanghai and Singapore

<u>Analyte</u> *	<u>Case # subjects</u>	<u>Control # subjects</u>	<u>OR</u> **	<u>95% CI</u>	<u>P</u>
sCD27					
Median [^] (P25,P75)	107 [¥] (84,133)	92 (73, 115)			
Q1	32	55	1.00	Ref.	Ref.
Q2	43	54	1.60	0.83-3.09	0.16
Q3	47	55	1.94	0.98-3.83	0.055
Q4	96	54	4.45	2.25-8.81	0.00002
p-trend					0.000005
sCD30					
Median [^] (P25,P75)	38398 [¥] (29206,49991)	31458 (24814,38890)			
Q1	27	55	1.00	Ref.	Ref.
Q2	39	54	1.74	0.85-3.58	0.13
Q3	45	55	1.86	0.94-3.67	0.074
Q4	107	54	5.15	2.62-10.12	0.000002
p-trend					0.0000002

* Quartile cut-offs for sCD27 (Q1 = 73 U/ml; Q2 = 73-91.5 U/ml; Q3 = 91.5-115 U/ml; Q4 > 115 U/ml) and sCD30 (Q1 = 24814 pg/ml; Q2 = 24814-31458.50 pg/ml; Q3 = 31458.50-38890 pg/ml; Q4 > 38890 pg/ml) based on distributions in all study controls

** Models further adjusted for age (continuous) and ever smoking

[^] Distributions of sCD27 (U/ml) and sCD30 (pg/ml) levels in all study cases and controls

[¥] P < 0.05 from Wilcoxon signed-rank test comparing median analyte levels in cases and controls

Table 3

Associations between sCD27 and risk of NHL stratified by the time period between blood collection and diagnosis in a pooled analysis of three prospective cohorts of Chinese men and women in Shanghai and Singapore

<u>Time</u>	<u>Median sCD27 (U/ml), Cases</u>	<u>Tertile 1</u>	<u>Tertile 2</u>	<u>Tertile 3</u>	<u>P-trend</u>
<u>Median</u>					
< 7 years	109	OR (95% CI) * Cases/Controls 22/42	1.73 (0.84-3.55) 26/31	4.71 (2.29-9.70) 55/30	<0.0001
> 7 years	107	OR (95% CI) Cases/Controls 19/31	1.73 (0.89-3.38) 38/43	2.65 (1.36-5.19) 58/41	0.005
P-het[†]					0.20
<u>Tertiles</u>					
0- 4.5 years	113	OR (95% CI) Cases/Controls 18/29	1.24 (0.54-2.82) 15/24	4.16 (1.88-9.20) 38/18	0.0001
>4.5- <10 years	109	OR (95% CI) Cases/Controls 11/22	2.13 (0.95-4.76) 24/21	3.48 (1.56-7.76) 42/34	0.002
10 years	103	OR (95% CI) Cases/Controls 12/22	1.99 (0.89-4.47) 25/29	2.91 (1.28-6.61) 33/19	0.01
P-het[†]					0.80

* ORs, 95% confidence intervals, and p-values estimated using polytomous logistic regression with case groups defined by median and tertiles of time between blood collection and diagnosis and using all controls as reference group. Models adjusted for gender, age (continuous), study, year of blood collection, and ever smoking

[†]Test for difference between parameter estimates associated with the highest tertile across case strata within polytomous logistic regression model

Table 4

Associations between sCD30 and risk of NHL stratified by the time period between blood collection and diagnosis in a pooled analysis of three prospective cohorts of Chinese men and women in Shanghai and Singapore

<u>Time</u>	<u>Median sCD30 (pg/ml), Cases</u>	<u>Tertile 1</u>	<u>Tertile 2</u>	<u>Tertile 3</u>	<u>P-trend</u>
<u>Median</u>	<u>sCD30</u>				
< 7 years	42925	OR (95% CI) * 1.00 (ref)	2.00 (0.90-4.45)	5.01 (2.42-10.39)	<0.0001
		Cases/Controls 12/28	24/37	67/38	
> 7 years	35301	OR (95% CI) 1.00 (ref)	1.32 (0.70-2.49)	2.69 (1.47-4.94)	0.0007
		Cases/Controls 29/44	34/38	52/33	
P-het[†]					0.14
<u>Tertiles</u>					
0- 4.5 years	45035	OR (95% CI) 1.00 (ref)	1.49 (0.58-3.84)	4.48 (1.96-10.25)	0.00004
		Cases/Controls 9/19	14/22	48/30	
>4.5- <10 years	37669	OR (95% CI) 1.00 (ref)	1.14 (0.54-2.43)	2.61 (1.33-5.13)	0.002
		Cases/Controls 19/28	18/29	40/20	
10 years	35301	OR (95% CI) 1.00 (ref)	2.12 (0.94-4.75)	3.83 (1.72-8.56)	0.001
		Cases/Controls 13/25	26/24	31/21	
P-het[†]					0.49

* ORs, 95% confidence intervals, and p-values estimated using polytomous logistic regression with case groups defined by median and tertiles of time between blood collection and diagnosis and using all controls as reference group. Models adjusted for gender, age (continuous), study, year of blood collection, and ever smoking

[†]Test for difference between parameter estimates associated with the highest tertile across case strata within polytomous logistic regression model