Incidental lymphopenia and mortality: a prospective cohort study

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ABSTRACT

BACKGROUND: It is unknown if incidental lymphopenia detected in the general population is associated with higher all-cause and cause-specific mortality. We aimed to identify the associations between lymphopenia and all-cause and cause specific mortality.

METHODS: In a prospective cohort study, we examined and followed participants enrolled in the Copenhagen General Population Study between November 2003 and April 2015. In our analysis, we modelled risks using Cox proportional hazards regression for 3 groups: participants with a lymphocyte count below the 2.5th percentile; those with a lymphocyte count at or between the 2.5th and 97.5th percentiles (reference cate-

gory); and those with a lymphocyte count above the 97.5th percentile.

RESULTS: The cohort included 108 135 participants with a median age of 68 years. During a median follow-up of 9 (interquartile range [IQR] 0–14) years, 10 372 participants died. We found that participants with lymphopenia (lymphocyte count $< 1.1 \times 10^9$/L) compared with those with a lymphocyte count in the reference range ($1.1–3.7 \times 10^9$/L) had higher mortality with multivariable adjusted hazard ratios (HRs) of 1.63 (95% confidence interval [CI] 1.51–1.76) for all causes, 1.67 (95% CI 1.42–1.97) for nonhematologic cancers, 2.79 (95% CI 1.82–4.28) for hematologic cancers, 1.88 (95% CI 1.61–2.20) for

cardiovascular diseases, 1.88 (95% CI 1.55–2.29) for respiratory diseases, 1.86 (95% CI 1.53–2.25) for infectious diseases, and 1.50 (95% CI 1.19–1.88) for other causes. For all-cause mortality, the highest absolute 2-year risks of death were observed in women (61%) and men (75%) who smoked and were aged 80 years or older with lymphocyte counts less than 0.5×10^9/L. Participants with a lymphocyte count higher than the reference category had increased mortality (adjusted HR 1.17, 95% CI 1.04–1.31).

INTERPRETATION: We found that lymphopenia was associated with an increased risk of all-cause and cause-specific mortality.

Lymphopenia in an otherwise healthy person is typically discovered when doing routine blood counts. In Denmark, patients with incidental lymphopenia are usually not referred for further examination because the mortality implications are unknown. In general, predictors of mortality are highly valued in everyday clinical practice because they help identify patients who may benefit from additional medical attention.

Observational studies have shown lymphopenia to be associated with risk of cardiovascular disease,[1–5] cancer,[6–11] liver disease (including alcohol abuse disorders)[12,13] and systemic autoimmune disease.[14–16] In a 2018 study involving 98 344 participants from the general population of Denmark, we found that lymphopenia was associated with a high risk of infectious disease.[17] The risk of all-cause mortality for patients with incidental lymphopenia is not known. This study aimed to identify the associations

between lymphopenia and all-cause and cause-specific mortality.

Methods

We followed the reporting standards outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[18]

Study design and participants

Participants who were white, of Danish descent and aged 20–100 years were eligible if they participated in the Copenhagen General Population Study,[17] a prospective cohort study that enrolled participants from November 2003 to April 2015 from suburbs in and around Copenhagen, Denmark.[19–21] Further details can be found in Appendix 1, Supplementary Methods,

available at www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj. 191024/-/DC1. We enrolled participants using the Danish Civil Registration System,[22] they answered a questionnaire about health and lifestyle (Appendix 2, available at www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj.191024/-/DC1), and each participant underwent a physical examination (Appendix 3, available at www.cmaj.ca/lookup/suppl/doi:10.1503/cmaj.191024/-/DC1) during which blood samples were drawn. All participants gave written, informed consent.

Blood samples were drawn on the day that participants were examined and white blood cell counts were measured immediately using the ADVIA 120 Hematology system. Precision of the measurements was monitored daily: day-to-day coefficient of variation was typically 2%–5% (in the range of $1.5–1.7 \times 10^9$/L lymphocytes). We notified participants and recommended further medical evaluation if their blood sample had a total leukocyte count above 50×10^9/L (indication of a high risk of leukemia).

We used the Danish Civil Registration System to access dates of death for all participants who died before Apr. 19, 2018. The register holds information on all individuals alive and living in Denmark and is continuously updated with vital statistics data.[23] We defined all-cause mortality as death regardless of cause, which was further classified according to the cause of death using the Danish Register of Causes of Death.[22] Using the *International Statistical Classification of Diseases and Related Health Problems*, *10th Revision* (ICD-10), deaths were classified as non-hematologic cancer mortality, hematologic cancer mortality, cardiovascular mortality, respiratory mortality and infectious disease mortality (Appendix 1, Supplementary Table S1). The remaining causes of death were classified as other mortality. In Denmark, physicians may list several causes of death on the death certificate. Therefore, some individuals had causes of death in more than 1 category. Information on cause of death was available until Dec. 31, 2015, because this was the latest update available on causes of death.

Covariates

We chose covariates a priori based on a 2014 WHO report evaluating the most common risk factors for all-cause mortality.[24] We also included covariates associated with lymphopenia.[13,25–27] Specific information on covariates associated with lymphopenia can be found in Appendix 1, Supplementary Methods. Missing values (0.9% on average; Appendix 1, Supplementary Table S2) were imputed.

Statistical analysis

We conducted the statistical analysis using Stata version 13.1. All statistical tests were 2-sided. Differences in baseline characteristics were assessed using Cuzick's extension to the Wilcoxon rank sum test for continuous variables or logistic regression for categorical variables.

We modelled risks of all-cause and cause-specific mortality separately using Cox proportional hazards regression, with lymphocyte counts categorized into the following 3 groups: participants were considered to have lymphopenia if their lymphocyte count was below the 2.5th percentile; a reference category was created with a lymphocyte count at or between the 2.5th and 97.5th percentiles; and participants were considered to have lymphocytosis if their lymphocyte count was above the 97.5th percentile. We used restricted cubic splines[28] for presentation of results from the Cox models. When we analyzed all-cause mortality, we considered follow-up to begin at the date of examination and end on the date of death due to any cause, emigration or Apr. 19, 2018, whichever came first. When we analyzed cause-specific mortality, we considered follow-up to end on the date of cause-specific death, emigration or Dec. 31, 2015, whichever came first. We accounted for competing risk of death in analyses of cause-specific mortality by calculating subhazard ratios using Fine and Gray's method.[29]

We plotted cumulative mortality using a multivariable-adjusted Fine and Gray regression model of all-cause mortality, taking emigration as a competing event into consideration. This method allows calculation of cumulative incidence not influenced by emigration. We tested for interactions by using likelihood ratios.

We used age as the underlying time scale (= age adjusted) in the Cox and Fine and Gray models, and the multivariable models were additionally adjusted for birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, plasma cholesterol, plasma triglycerides, education, income and leisure-time physical activity.

Appendix 1 (Supplementary Methods) contains information on how we handled missing data. We also adjusted the main analyses for regression dilution bias because this reduces the influence of biological and measurement variability of lymphocyte count on risk estimates. We calculated regression toward the mean[30] by using repeat measurements of lymphocyte counts in 5181 participants after a median of 10.5 (interquartile range [IQR] 9.9–10.8) years. We based the ratio on the median result value in the groups with lymphopenia and lymphocytosis, using the 2.5th and 97.5th percentile cut-offs for lymphocyte counts at the first date of examination (calculated as 0.68).

Because lymphocyte count declines with increasing age[31,32] (Appendix 1, Supplementary Figure S3), we redefined lymphopenia by calculating the age-adjusted 2.5th percentile for lymphocyte count for each 10-year age group.

Subclinical, but undiagnosed disease, present on the day of examination could affect both lymphocyte count and mortality. Therefore, we subdivided follow-up time into 3 time intervals of 0–4, 4–8 and more than 8 years, to assess if follow-up time affected the association between lymphopenia and all-cause mortality. We excluded all individuals with either diagnosed comorbidity, as defined by the Charlson Comorbidity Index, or with results that deviated from reference values for routine blood tests (Appendix 1, Supplementary Methods). When comparing risk estimates, we used the *Z*-test described by Bland and Altman.[33] In addition, we adjusted the main analysis of all-cause mortality for the Charlson Comorbidity Index. We created a prediction algorithm to show participants' absolute 2-year risk of

all-cause mortality using the Framingham Risk Score as inspiration[34] (Appendix 1, Supplementary Figure S11).

Ethics approval

This study was approved by a Danish ethical committee (H-KF-01–144/01).

Results

Table 1 presents baseline characteristics of 108 136 included participants from the Copenhagen General Population Study according to blood lymphocyte count; we present absolute differences between the 3 groups. Lymphocyte concentrations ranged from 0.1 to 125 × 10⁹/L and were almost normally distributed (Appendix 1, Supplementary Figure S4). A total of 61 people who were not white and not of Danish descent were excluded. No participants were lost to follow-up, and 456 emigrated and were excluded. Missing values were imputed; however, results

were similar without imputation. Complete population characteristics, numbers of missing information and explanations for missing values can be found in Appendix 1, Supplementary Table S2. During a median follow-up of 9 (IQR 0–14) years, 10 372 participants died before Apr. 19, 2018. A flowchart of participants is available (Appendix 1, Supplementary Figure 2).

After we adjusted for age and sex, the hazard ratio (HR) for all-cause mortality was 1.63 (95% confidence interval [CI] 1.51–1.76) in participants with lymphopenia and 1.47 (95% CI 1.31–1.65) in participants with lymphocytosis when compared with participants with lymphocytes in the reference range (Figure 1). In the multivariable-adjusted model, we determined that the corresponding HRs were 1.63 (95% CI 1.51–1.76) and 1.17 (95% CI 1.04–1.31), respectively. After adjustment for regression dilution bias the corresponding HRs were also significant (Appendix 1, Supplementary Figure S5).

Figure 2 presents the cumulative mortality in participants with lymphopenia (lymphocyte count < 1.1 × 10⁹/L) versus those

Table 1: Baseline characteristics of participants from the Copenhagen General Population Study,[17] by blood lymphocyte count

Characteristic	No. (%) of participants*		
	Participants with lymphopenia (lymphocyte count < 1.1 × 10⁹/L) n = 2647	Reference group (lymphocyte count = 1.1–3.7 × 10⁹/L) n = 102 879	Participants with lymphocytosis (lymphocyte count > 3.7 × 10⁹/L) n = 2610
Blood lymphocyte count, × 10⁹/L; median (IQR)	1.0 (0.1–1.09)	2.1 (1.1–3.69)	4.0 (3.7–125)
Age, yr; median (IQR)	68 (58–77)	58 (48–67)	58 (48–66)
Male sex	1465 (55)	46 145 (45)	986 (38)
Ever smoked	1529 (58)	59 102 (57)	1951 (75)
Cumulative smoking, pack-years;† median (IQR)	16 (5–32)	15 (6–30)	25 (13–40)
Alcohol consumption > 168 g/wk for men, > 84 g/wk for women‡	1081 (41)	40 261 (39)	938 (36)
Body mass index, kg/m²; median (IQR)	25 (23–28)	26 (23–28)	27 (24–30)
Plasma C-reactive protein, mg/L; median (IQR)	1.6 (1.0–3.4)	1.4 (0.9–2.2)	1.8 (1.2–3.4)
Blood neutrophil count, × 10⁹/L; median (IQR)	3.7 (2.9–4.8)	4.0 (3.3–4.9)	5.0 (4.1–6.1)
Any recent infection	136 (5)	3969 (4)	139 (5)
Diabetes	164 (6)	3717 (4)	125 (5)
Systolic blood pressure, mm Hg; median (IQR)	142 (128–158)	140 (126–155)	141 (128–158)
Plasma cholesterol, mmol/L; median (IQR)	5.3 (4.5–6.0)	5.5 (4.8–6.3)	5.8 (5.1–6.5)
Plasma triglycerides, mmol/L; median (IQR)	1.2 (0.9–1.8)	1.4 (0.9–2.0)	1.8 (1.2–2.7)
High income§	1288 (49)	65 389 (64)	1499 (57)
High leisure-time physical activity¶	1257 (48)	53 066 (52)	1053 (40)
More than 12 years of education	2063 (78)	81 132 (79)	1842 (71)
Comorbidity**	2095 (79)	55 635 (54)	1819 (70)

Note: IQR = interquartile range.
*Unless specified otherwise.
†Only ever smokers.
‡Values from the The Danish State Health Authority (latest national recommendations available at www.sst.dk/da/Udgivelser/2018/Forebyggelsespakke-Alkohol [in Danish]).
§Greater than or equal to 400 000 Danish krone/household.
¶More than 4 hours of physical activity a week.
**Defined as participants with any comorbidity or with routine blood test results outside the reference range.
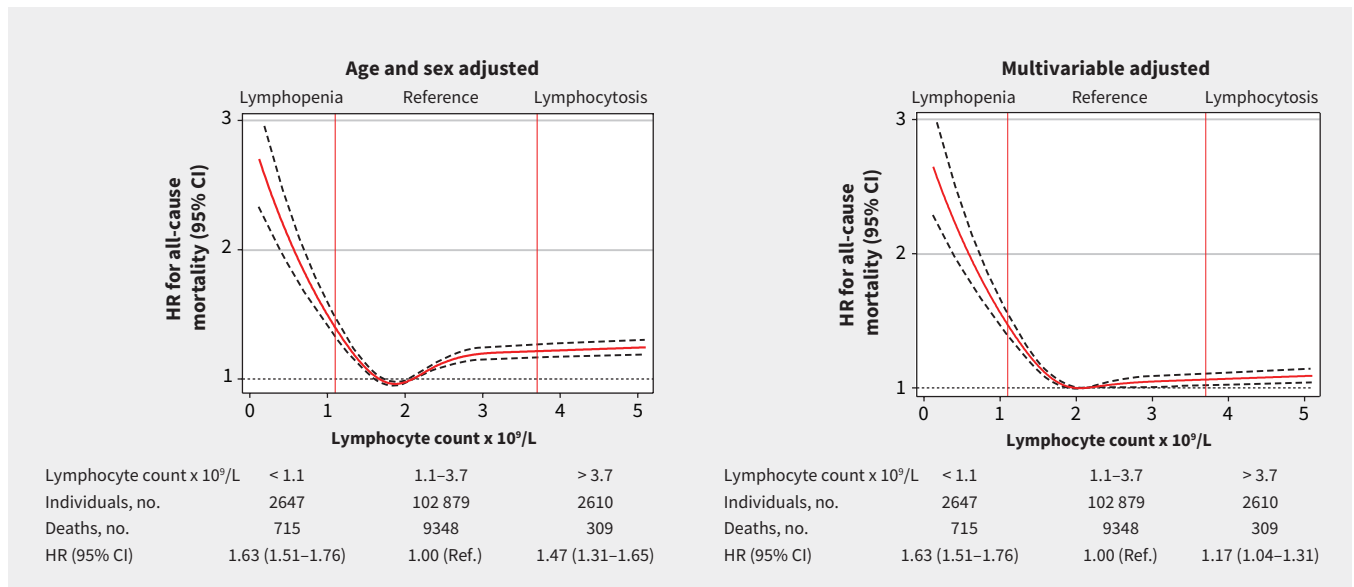
Figure 1: All-cause mortality as a function of lymphocyte count in participants from the Copenhagen General Population Study.[17] Solid red lines are HRs and broken black lines indicate 95% CIs based on fitting of cubic splines to risk estimates obtained using Cox Proportional Hazards regression. We used age as the underlying time scale; we also adjusted the multivariable adjusted model for birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, level of plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, level of plasma cholesterol, level of plasma triglycerides, education, income and leisure-time physical activity. We set the median lymphocyte value (2.1 × 10⁹/L) as the reference for the continuous model. When categorizing lymphocyte counts, we considered lymphopenia to be present when a participant had a lymphocyte count lower than 1.1 × 10⁹/L (2.5th percentile); the reference category was lymphocyte count between 1.1 and 3.7 × 10⁹/L (2.5th to 97.5th percentile). We considered lymphocytosis to be present when a participant had a lymphocyte count above 3.7 × 10⁹/L (97.5th percentile). Note: Estimates for all-cause mortality in individuals with lymphopenia are identical in the 2 models. CI = confidence interval, HR = hazard ratio.

with a lymphocyte count in the reference range (1.1–3.7 × 10⁹/L) (log-rank < 0.001).

We found that the risk estimates for all-cause mortality in participants with lymphopenia compared with those with lymphocyte counts in the reference range were more pronounced in participants at or below age 70 years than in those above age 70 years (*p* for interaction < 0.001) (Figure 3). This was true regardless of whether lymphopenia was defined as participants having a lymphocyte count of less than 1.1 × 10⁹/L, the 2.5th percentile for the entire population or whether the cut-off was defined separately for each 10-year age group (Figure 3 and Appendix 1, Supplementary Figure S6).

In analyses of mortality, stratified for the covariates associated with lymphopenia at baseline (Appendix 1, Supplementary Figure S7), we found a significant interaction between lymphocyte count and age (*p* < 0.001), and lymphocyte count and leisure time physical activity (*p* = 0.004); these significant *p* values indicate that risk of all-cause mortality by lymphopenia was lower in those participants older than 70 years of age compared with those aged 70 years or younger and in those with leisure time physical activity of less than 4 hours versus those with 4 or more hours per week.

A total of 7029 participants died before Dec. 31, 2015 (non-hematologic cancers, *n* = 2663; hematologic cancers, *n* = 218; cardiovascular diseases, *n* = 2005; respiratory diseases, *n* = 1342; infectious diseases, *n* = 1299; and other causes, *n* = 1207) during a median follow-up of 7 (IQR 0–12) years.

In multivariable-adjusted models, we found that participants with lymphopenia had higher mortality than those with lympho-

cyte counts in the reference range for all causes of mortality: HR for nonhematologic cancers 1.67 (95% CI 1.42–1.97), HR for hematologic cancers 2.79 (95% CI 1.82–4.28), HR for cardiovascular diseases 1.88 (95% CI 1.61–2.20), HR for respiratory diseases 1.88 (95% CI 1.55–2.29), HR for infectious diseases 1.86 (95% CI 1.53–2.25) and HR for other causes 1.50 (95% CI 1.19–1.88) (Figure 4). Our findings were similar after accounting for competing risks by calculation of subhazard ratios. In addition, participants with lymphocytosis had a higher mortality for hematologic cancers (HR 4.54 (95% CI 2.85–7.23), which is consistent with lymphocyte count being a hematologic index.

We determined that redefining lymphopenia using different cut-off values produced mortality estimates that were similar to our main findings (Appendix 1, Supplementary Figure S8). When we subdivided follow-up time (Figure 5), the multivariable adjusted HR for mortality more than 8 years after the day of examination was 1.50 (95% CI 1.27–1.77). To further exclude undiagnosed comorbidity as the explanation for our results, we excluded all participants with either diagnosed comorbidity or with results from routine blood tests that deviated from reference values on the day of examination (*n* = 48 567) (Appendix 1, Supplementary Figure S9). The risk estimates were similar to those obtained when evaluating all participants (*p* value for difference = 0.20). We obtained similar results for all-cause mortality after adjusting the main analysis with the Charlson Comorbidity Index (Appendix 1, Supplementary Figure S10).

Our prediction algorithm found the combination of lymphopenia with other risk factors categorized participants in groups
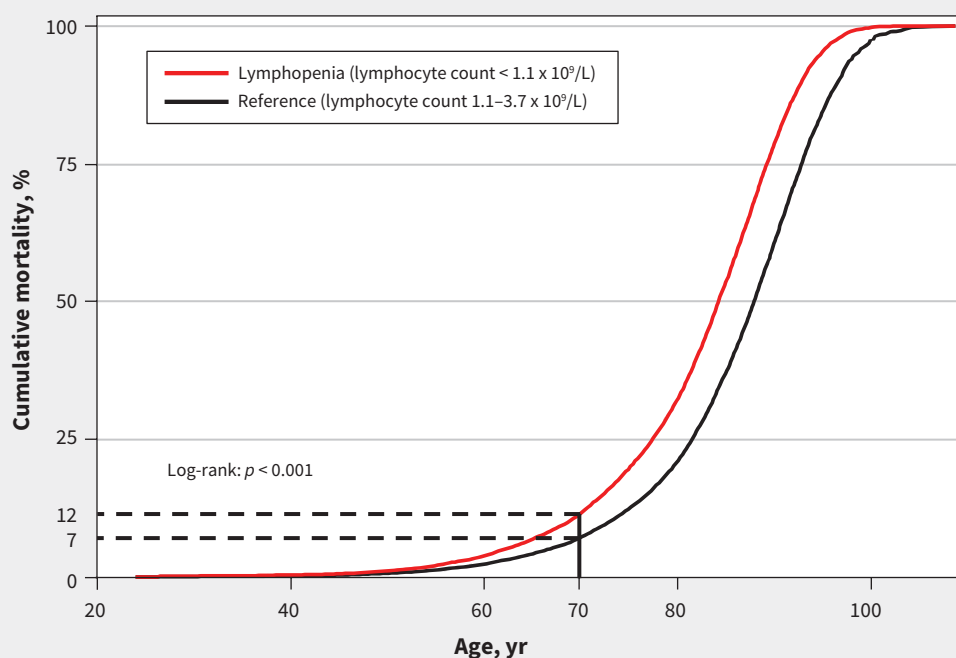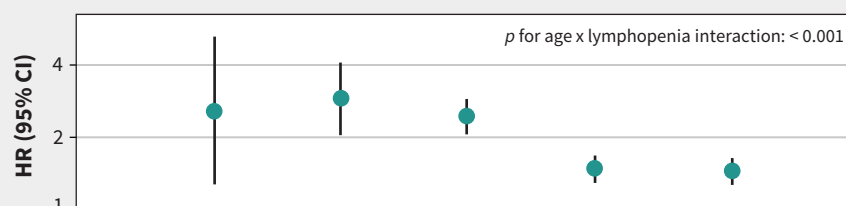
Figure 2: Cumulative all-cause mortality as a function of lymphopenia in participants from the Copenhagen General Population Study.[17] We used the Fine and Gray method,[29] taking emigration (*n* = 456) as a competing event into consideration to calculate cumulative all-cause mortality for participants with lymphopenia (lymphocyte count < 1.1 × 10⁹/L) and those with lymphocyte counts in the reference range (1.1–3.7 × 10⁹/L). We used age as the underlying time scale and additionally adjusted the model for birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, level of plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, level of plasma cholesterol, level of plasma triglycerides, education, income and leisure-time physical activity. At 70 years of age, the cumulative mortality for participants with lymphopenia and those with lymphocyte counts in the reference range were 12% and 7%.

Age, yr	≤ 50	> 50–60	> 60–70	> 70–80	> 80
Individuals, no.	31 374	26 418	27 499	15 395	4840
Deaths, no.	345	964	2437	3729	2588
Deaths/100 000 person years	120	407	1040	3075	15 198
Cut-off for lymphopenia, x 10⁹/L, 2.5th percentile in the study population	1.1	1.1	1.1	1.1	1.1
HR	2.57	2.87	2.42	1.46	1.43
(95% CI)	(1.27–5.22)	(2.03–4.06)	(2.04–2.87)	(1.29–1.67)	(1.26–1.63)

Figure 3: All-cause mortality as a function of lymphopenia stratified by age for participants from the Copenhagen General Population Study.[17] We used age as the underlying time scale and additionally adjusted the multivariable model by birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, level of plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, level of plasma cholesterol, level of plasma triglycerides, education, income and leisure-time physical activity. Participants were categorized in 10-year age groups, and we considered those with a lymphocyte count lower than 1.1 × 10⁹/L to have lymphopenia. Note: *p* value for interaction was calculated according to a likelihood ratio test. CI = confidence interval, HR = hazard ratio.
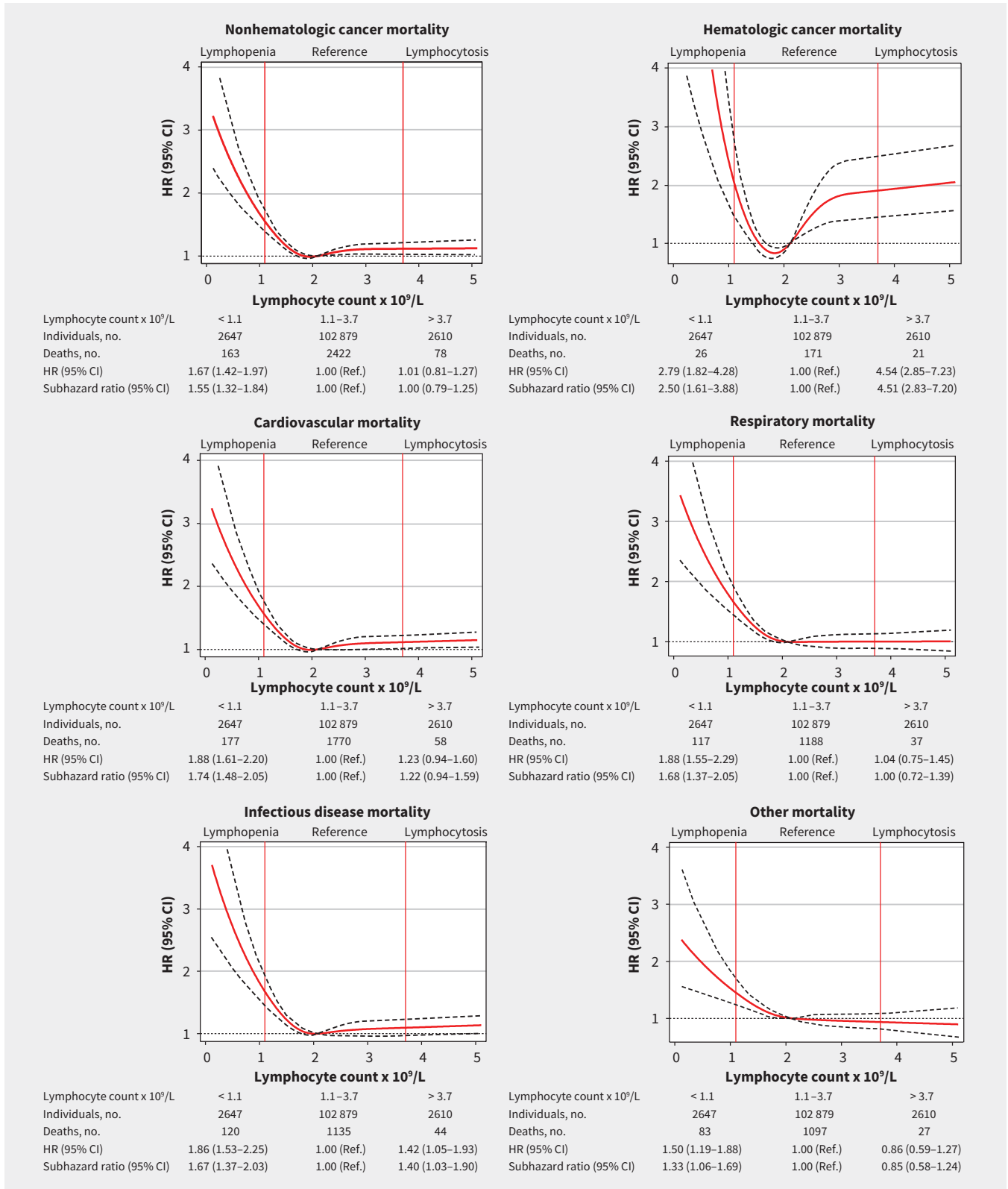
Figure 4: Cause-specific mortality as a function of lymphocyte count for participants from the Copenhagen General Population Study.[17] Solid red lines are multivariable adjusted HRs and broken black lines indicate 95% CIs based on fitting of cubic splines to risk estimates obtained using Cox Proportional Hazards regression. We obtained subhazard ratios using a Fine and Gray regression. Age was used as the underlying time scale and we additionally adjusted the multivariable model by birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, level of plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, level of plasma cholesterol, level of plasma triglycerides, education, income and leisure-time physical activity. We set the median lymphocyte value (2.1 × 10⁹/L) as the reference for the continuous model. The sum of cause-specific deaths exceeds the total number of deaths because participants may have had several causes of death listed on their death certificate. Note: CI = confidence interval, HR = hazard ratio, Ref. = reference category.
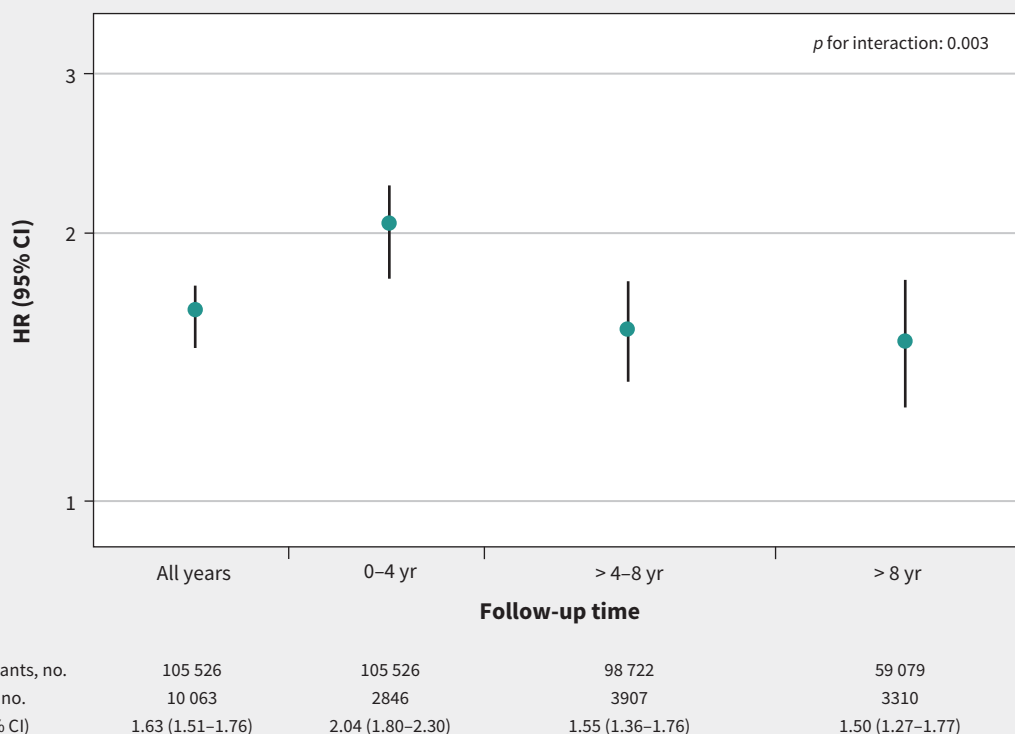
Figure 5: All-cause mortality stratified by follow-up interval for participants from the Copenhagen General Population Study.[17] We compared participants with lymphopenia (lymphocyte count < 1.1 × 10⁹/L) with those who had a lymphocyte count in the reference range (1.1–3.7 × 10⁹/L). We used age as the underlying time scale and additionally adjusted the multivariable model by birth year, sex, smoking status, cumulative smoking in pack-years, alcohol consumption, body mass index, level of plasma C-reactive protein, blood neutrophil count, recent infections, diabetes, systolic blood pressure, level of plasma cholesterol, level of plasma triglycerides, education, income and leisure-time physical activity. We subdivided follow-up time into 3 time intervals, and we included participants in more than 1 time interval if total follow-up was more than 4 years. Note: *p* for interaction was calculated according to a likelihood ratio test. CI = confidence interval, HR = hazard ratio.

with absolute 2-year risks of all-cause mortality from below 1% to the highest in women (61%) and men (75%) who smoked and were aged 80 years or older with lymphocyte counts less than 0.5×10^9/L.

Interpretation

In this prospective cohort study involving participants from the general population in Denmark, we found that lymphopenia was associated with 1.6-fold increase in all-cause mortality and with a 1.5 to 2.8-fold increased in risk of mortality owing to nonhematologic cancer, hematologic cancer, cardiovascular disease, respiratory disease, infections and other causes.

Our study showed that participants with lymphopenia were at high risk of dying from any cause, regardless of any other risk factor for all-cause mortality including age. This might be due to a reduced immune surveillance, which makes these patients less able to survive potentially deadly diseases. Lymphopenia could also be a more passive marker of general frailty that confers high risk of death from any cause. Older age is associated both with decreasing lymphocyte count,[31,35]

which we found in this study, and with mortality. Although the reason for the age-associated decline in lymphocyte count is not known, possible explanations may include age-related thymic involution, which leads to a change in the composition of lymphocyte subsets with age,[36–38] and, as a consequence, a change in overall immune competence.[39,40] The higher mortality risk shown among those with lymphopenia who were younger than 70 years of age compared with those aged 70 years or older suggests that factors other than age might contribute to the high mortality risk, such as poor immune surveillance, iatrogenic causes (i.e., medications) and blood donations or transfusions.

Using the same cohort, we recently determined that lymphopenia was associated with a 1.4-fold risk of admission to hospital because of infectious disease and a 1.7-fold risk of infection-related death.[17] Previous studies have focused mainly on lymphopenia and risk of cardiovascular death, both in outpatient settings and in hospital populations.[1–5] In support of our findings, these studies reported that low lymphocyte count was associated with risk of death from acute or chronic heart failure.[1–5] Lymphopenia has also been associated with short overall survival

and short progression-free survival in patients with breast cancer,[9] sarcomas and non-Hodgkin lymphoma.[8]

To illustrate the predictive capacity of the lymphocyte count, we determined absolute 2-year risks of all-cause mortality in participants from a general population. We found that risk estimates were highly affected by the lymphocyte count, indicating that a selected group (e.g., smokers older than 80 years with a low lymphocyte count) might benefit from additional surveillance, although there is no evidence that this will decrease mortality.

Limitations

We could not clarify questions of causality. Participants may have had subclinical and potentially fatal disease on the day of examination, which might have affected their lymphocyte count and mortality risk. However, since the association between lymphopenia and high mortality remained for several years after participants were examined, this is unlikely to have biased our findings. Nonetheless, some of the observed association may be explained by residual confounding. Iatrogenic causes of lymphopenia such as medication and blood donations could not be evaluated because data were not available. A limitation to the analyses of cause-specific mortality was the potential misclassification of causes, because we obtained the data directly from the national Danish Register of Causes of Death without adjudication. However, misclassification of cause of death would be biased toward the null hypothesis because the attending physician is not likely to have known the lymphocyte count measured in this research cohort. In population-based studies, participants with comorbidities are less likely to participate, which generates a healthy participant bias, but this is more likely to bias our results toward the null hypothesis and probably does not explain our observed association between lymphopenia and high mortality. Lastly, the generalizability of the results may be limited because our study involved only participants who were white and of Danish descent. It remains to be seen if lymphopenia increases risk of mortality in nonwhite populations.[41,42]

Conclusion

In this prospective cohort study, we found that incidental lymphopenia was associated with a 1.6-fold increase in risk of all-cause mortality and a 1.5 to 2.8-fold increased risk of cause-specific mortality. Using the absolute 2-year risks of all-cause mortality, physicians can identify high-risk individuals with lymphopenia (e.g., smokers aged more than 60 years) who might benefit from additional surveillance.

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