Raised levels of immunoglobulin G, A and M are associated with an increased risk of total and cause-specific mortality
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DOI:
10.1136/jech-2014-204345
Raised levels of immunoglobulin G, A and M are associated with an increased risk of total and cause-specific mortality: the Vietnam Experience Study

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ABSTRACT

Background Immunoglobulins (Ig) are essential for combating infectious disease. However, high levels are associated with a range of diseases and/or poor health behaviours, such as autoimmune diseases, chronic infection, HIV and excessive alcohol consumption. In the present analyses, we extend this body of work by examining whether higher levels of serum Ig G, A and M are associated with increased mortality risk.

Methods Participants were 4255 Vietnam-era, former US army personnel (the Vietnam Experience Study). From military service files, telephone interviews in 1983 and a medical examination in 1986, sociodemographic, and health data were collected. Contemporary morning fasted blood samples were taken from which IgG, IgA and IgM concentrations were determined. Mortality surveillance over 15 years gave rise to deaths ascribed to all-causes, cardiovascular disease mortality, all cancers combined mortality, external cause and ‘other’ causes (predominantly comprising deaths due to infectious disease). Cox proportional hazard models were utilised (predominantly comprising deaths due to infectious disease). Cox proportional hazard models were utilised to compute HRs per SD increase in Ig which were first adjusted for age and then additionally adjusting for a range of candidate confounders.

Results In multiply adjusted analyses, in general, the higher the immunoglobulin concentration, the greater the risk of death. Thus, IgA (HR=2.0 95% CI 1.47 to 2.73), IgM (HR=1.5 95% CI 1.11 to 1.91) and IgG (HR=5.8 95% CI 3.38 to 9.95) were positively related to all-cause mortality. Corresponding results for ‘other’ causes of mortality were 4.7 (2.64 to 8.19), 3.5 (2.29 to 5.45) and 33.4 (15.13 to 73.64).

Conclusions In the present study, high levels of Ig are associated with an elevated risk of death from total and ‘other’ causes, mainly infectious disease. High levels of Ig, particularly IgG, may signal subclinical disease.

Immunoglobulins (Ig) or antibodies are proteins secreted by white blood cells (B lymphocytes) which circulate in the body and tag, destroy and/or neutralise bacteria, viruses and other harmful or foreign materials (antigens). This is achieved by opsonising or coating foreign materials which marks them for destruction or neutralisation. Ig are present on the membrane surface of B cells and act as receptors for foreign materials. Binding of specific antigen to B-cell surface immunoglobulin stimulates the B cell to divide (clonal expansion) and for some of its progeny to differentiate into plasma cells. Plasma cells are factories that produce and secrete large amounts of antibody directed against the antigen that stimulated the original B cell.

Ig can be classified into five isotypes (IgM, IgD, IgG, IgA and IgE) with four subclasses of IgG and two of IgA. Each isotype has a range of different immune functions. IgM and IgD are the isotypes expressed on the surface of all naïve B lymphocytes (ie, cells with no previous antigen exposure) where they are the receptor for specific pathogens or antigens. IgM is the first isotype to be secreted in an antibody response. IgM is of low affinity for antigen but being pentameric can bind antigens with great avidity and it is the most potent antibody for activating complement to destroy the antigen target. When naïve B cells are activated they can switch from their original IgM immunoglobulin to one of the other isotypes. In addition, after B-cell activation, the structure of the immunoglobulin antigen binding site is refined to produce effector antibodies with a more precise and stronger fit, that is, higher affinity, to specific antigens. IgG is the most abundant immunoglobulin isotype in the human body and is usually of high affinity. This high affinity makes IgG particularly effective at neutralising the toxins secreted by bacteria and the docking molecules by which viruses enter our cells. IgA is secreted at the mucosal surfaces (eg, mouth, nose, gastrointestinal tract) and is the first line of defence against infection present at these surfaces to prevent colonisation by antigens. It can be measured in serum as well as saliva. IgE is present in the body at very low concentrations compared to the other isotypes but high levels are associated with parasitic infections such as worms. The bone marrow randomly generates a pool of billions of B cells each with antibody directed against a different antigen. Encounter with a new pathogen leads to clonal expansion of pathogen specific B cells and secretion of large amounts of antibody against the pathogen. The expansion of B cells and secretion of pathogen specific antibody is maintained for many years, if not for life, providing protection against reinfection.

Generally, having a robust antibody response to pathogens is important in terms of protection against infectious disease, and is considered a key indicator of health. For example, better protection against hepatitis B following vaccination is related to higher IgG levels. Similarly, whereas the chronic stress of caregiving in older age is associated with lower IgA levels in saliva, moderate exercise training can restore Ig levels reduced by stress to normal levels and slow the ageing process. However, high levels of Ig are also associated with disease and/or poor health behaviours. For example, high IgE levels are associated with the atopic diseases asthma
and eczema and high levels of IgE are positively associated with alcohol consumption among individuals with atopic asthma; higher IgA production in the bowel may also be part of the cause of inflammatory bowel disease. Certain types of kidney disease are associated with infiltration of specific IgG4-positive plasma cells and abnormalities of the IgA system. Liver diseases are thought to increase levels of IgG (autoimmune hepatitis), IgM (primary biliary cirrhosis) and IgA (alcoholic liver disease). Further, elevated IgE is associated with community-acquired pneumonia. Regarding cancer, the neoplastic transformation of plasma cells is usually associated with secretion of very large amounts of immunoglobulin (monoclonal protein) specific to that clone of plasma cells that may be benign (monoclonal gammopathy of undetermined significance, MGUS is present in 3% of people aged over 50 years or malignant (myeloma). In contrast, clonal expansions of B cells like chronic lymphocytic leukaemia suppress normal antibody production leading to low serum immunoglobulin levels and greatly increased risk of infection. High levels of antibodies can also indicate poorer infection control and immune dysregulation. For example, infection by HIV results in high levels of serum antibody although not of useful protective specificity, and higher levels of antibodies against latent viruses such as cytomegalovirus indicate viral reactivation.

Despite these antibody disease associations, studies of the relationship between circulating immunoglobulin levels and mortality risk are scarce. In the few studies conducted, an elevated immunoglobulin response to vaccination is associated with lower influenza-related morbidity and mortality, and impaired antibody responses in older adults are associated with increased pneumonia morbidity and mortality. Further, reactivation of latent viruses, indicated through increased specific antibodies can be a cause of mortality in immunocompromised patients. Nonetheless, to our knowledge, no studies have examined associations between levels of non-specific subtypes of antibody and cause-specific mortality.

As immunoglobulin levels are associated with a variety of health outcomes, it might be expected that they could relate to death from specific causes, even in the absence of a formal disease diagnosis during the lifetime of the individual. Diseases associated with high levels of immunoglobulin include liver disease, autoimmune diseases like rheumatoid arthritis, Sjögren’s disease and systemic lupus erythematosus, chronic infections like bronchiectasis, HIV and plasma cell dyscrasias like MGUS. Consequently, in the present analyses, we examine the association of immunoglobulin A, M and G with deaths from all-causes, cardiovascular disease (CVD), all cancers combined mortality, ‘other’ causes (corresponding to deaths that were not ascribed to CVD and cancer causes, this largely comprises infectious diseases) and external causes. In doing so, we took into account a range of covariates that might be considered confounders (eg, age, educational level etc.) due to their known associations with mortality and theoretically likely associations with Ig levels. We hypothesised that a positive relationship between Ig and mortality might arise, but did not speculate for which disease types this might be apparent.

METHOD

Participants

Members of the Vietnam Experience Study are comprise a subset of male military personnel drawn from approximately five million US Vietnam-era Army veterans whose service files were stored at the National Personnel Records Center. Participants were identified retrospectively from data gathered as part of the Vietnam Experience Study, a population-based study commissioned by the US congress to investigate the health consequences of the military experiences of Vietnam-era veterans. The Centers for Disease Control, Atlanta, had access to US Veteran Administration records and provided the present authors with a fully anonymised data set. Those who entered military service only between 1 January 1965 and 31 December 1971; served only one term of enlistment; served at least 16 weeks of active duty; earned a military specialty other than ‘trainee’ or ‘duty soldier’; at discharge from active duty had a military pay grade no higher than sergeant (in order to maximise the number sampled who took part in combat) and had not died during military duties were eligible for inclusion. Figure 1 shows the sampling of participants; the final cohort included 18 313 former military personnel. Ethical approval for the original study was given by the various relevant bodies, including the US Centers for Disease Control.

Data collection

Place of service and ethnicity was extracted from the military archives. Participants were both Vietnam and non-Vietnam veterans, and designated as being Vietnam veterans if they had served at least one tour of duty in Vietnam. All study members served in the US military during the Vietnam war era; we do not stratify our analyses by deployment status. The ethnicity of the study members was categorised as ‘white’, ‘black’ or ‘other’; the latter being Hispanics, Asians, Pacific Islanders, American Indians and Alaskan Natives. Figure 1 shows the numbers of participants followed up at each stage of data collection. From the telephone survey, educational grade achieved at school and household income in midlife were determined as measures of socioeconomic status. Frequency of alcohol consumption, cigarette smoking and marital status were ascertained, as well as whether or not they had a range of somatic physician-diagnosed health problems including hypertension, cancer, diabetes and coronary heart disease.

In 1986, a random sample of telephone interview respondents (N=6443) were invited to attend a 3-day medical examination at a military facility in Albuquerque, New Mexico; 4462 men who were representative of the original cohort attended (69.3% of those invited). The mean age at medical examination was 38.3 years (range 31.1–49.0). The final number of participants with complete data after the medical examination and available for the present analyses was 4255. This group represents 23.3% of persons originally enrolled in the study.

Serum immunoglobulin levels were measured from a fasted blood sample by nephelometry after an immunoprecipitation reaction using anti-IgA, anti-IgM or anti-IgG antibodies using the Beckman Immunochemistry System (Beckman Coulter Inc, Brea, California, USA). The nephelometer measures the rate of light scatter formation from the immune-precipitin reaction. When brought into contact with their respective antigens in solution, anti-IgA, anti-IgM and anti-IgG, produce a peak rate signal proportional to the increase in light scatter produced by the antigen-antibody reaction.

Total cholesterol was determined using a Kodak Ektachem 700 autoanalyser. All laboratory assays were assured by using bench and blind repeat controls run for one in 20 randomly chosen samples; the correlation coefficients between first and repeat samples exceeded 0.98. Bench controls yielded intra-assay and interassay coefficients of variation all <10%. Blood pressure, while seated, was measured twice in the right arm using a standard mercury sphygmomanometer;
and averaged. Height and weight, measured using standard
protocols, were used to calculate body mass index (BMI; kg/m²).

The vital status of men postmedical examination was ascer-
tained until 31 December 2000 by cross-checking and combin-
ing data from a variety of mortality databases supplied by the
US army, the Veterans Administration, the Social Security
Administration, the Internal Revenue Service and the National
Center for Health Statistics (NCHS). Investigators manually
reviewed the matches from each data source separately and clas-
sified the matches as true, false or questionable, for further
details see. Cause-of-death codes were obtained from NDI Plus, a
national mortality database with cause-of-death information.
Where cause of death codes were not available from the mortal-
ity database, CDC investigators obtained official copies of death
certificates, which were then coded by an experienced nosologist
at the CDC’s NCHS. Cause-of-death was classified via the
International Classification of Diseases (ICD) revision in place at
the time of death: the Ninth Revision (ICD-9) for deaths
between 1 January 1979 and 31 December 1998 and the Tenth
Revision (ICD-10) for deaths between 1 January 1999 and 31
December 2000. Mortality due to major CVD was classified
using ICD codes: ICD-9 390-434436-448, and ICD-10
I00-I78 which comprised: acute rheumatic fever; chronic
rheumatic heart diseases; hypertensive diseases; ischaemic heart
diseases; pulmonary heart disease and diseases of pulmonary cir-
culation; other forms of heart disease; cerebrovascular diseases;
diseases of arteries, arterioles and capillaries. The CVD mortality
variable thus encompasses death from a variety of disorders;
the bulk of the deaths were from ischaemic heart disease. Mortality
from all cancers was classified using the ICD codes:
ICD-9 140-239, ICD-10 C00-D48 which include malignant
neoplasms of all specified areas, Hodgkin lymphoma,
non-Hodgkin lymphoma, leukaemia, multiple myeloma, immu-
noproliferative neoplasms and other malignant neoplasms of
unspecified areas. Of the cancers, the most frequent cause of
death (N=19) was from malignant neoplasms of the trachea,
bronchus and lung. External causes of death included those
coded as ICD-9 E800-E999, ICD-10 V01-Y8, including acci-
dents, suicide and homicide. Finally, ‘other’ causes of death
denotes total mortality minus the above classifications, and con-
sisted largely of death from viral or bacterial infection (N=31).
A00-B99, excluding infection with HIV (N=12), ICD-9 042-044, ICD-10 B20-B24 and from liver disease (N=12), ICD-9 571, ICD-10 K70,K73-K74.

**Statistical analysis**

Owing to their skewed distribution, immunoglobulin values were log-transformed and continuous logged values were used in all analyses. It was confirmed that the proportional hazards assumption had not been violated: partial residuals were all randomly distributed and mortality curves did not cross in Kaplan-Meier plots of Ig levels above and below the mean. Cox’s proportional hazard regression was used to compute HRs with accompanying CIs to summarise the relationships between Ig and mortality, first in age-adjusted analyses. Separate multivariable analyses of the relationships between Ig A, G, and M and mortality were then run, additionally adjusting for all covariates (place of service, ethnicity, education level, marital status, alcohol consumption, smoking, household income, BMI, cholesterol, systolic blood pressure, and physical illness diagnosis (cancer, diabetes and heart disease)). These covariates were chosen a priori as they have all been associated with mortality in this data set and others. The statistical package used was IBM SPSS V21.

**RESULTS**

**Sample characteristics**

The arithmetic mean (SD) serum IgA, IgM and IgG values for the whole sample were 227.1 (102.17), 135.3 (72.66) mg/dL and 1109.7 (288.54), respectively, with ranges of 1–1340, 21–1115 and 338–4360, respectively. Norms for IgA, IgM and IgG in serum using this methodology are 82–453, 46–304, and 751–1560 mg/dL, respectively, based on a sample of healthy male and female adults in the USA, although it is recommended that each laboratory should establish its own reference intervals. The characteristics of the sample are summarised in table 1 (a table depicting the association between Ig and potential confounding variables is given as online supplementary material).

**Ig and mortality risk**

Table 2 shows the correlations between the three immunoglobulin groups.
During the 15 years of follow-up there were 236 deaths. Age-adjusted Cox regression models conducted separately for each immunoglobulin showed associations between IgA, IgM and IgG, and all-cause mortality, such that higher levels of IgA, IgM and IgG were associated with an increased risk of mortality per SD increase in Ig. Table 3 shows the age-adjusted and multiply adjusted HR. No statistically significant associations emerged for CVD or cancer mortality, although effect sizes for IgA and IgG were around 1.5–2 times the risk of mortality per SD increase in Ig. There were also no statistically significant effects for external causes of death, although effect sizes for IgA and IgG were around 1.1–1.6 times the risk of mortality. However, for ‘other’ causes of death, IgA, IgM and IgG, were significantly associated with risk of death. It is noteworthy that using log-transformed antibody levels, immunoglobulin (per SD increase) is related to an increased mortality risk.

In the multiply adjusted models, shown in table 3, higher serum IgA, IgM and IgG levels were again associated with greater all-cause mortality. Thus, for every SD increase in immunoglobulin levels, there is a 1.5–5.8 times increased risk of mortality, depending on which immunoglobulin is being considered. Higher all-cause mortality was also associated with not being married, being non-white, smoking, having a physical illness, lower household income in midlife, lower educational level achieved and higher alcohol consumption. These covariates therefore account for the reduction in HR in the multiply adjusted models. Those with the greatest impact were being in the lowest income group, never being married or now divorced/separated, and being non-white, which all had HRs of 2.0 or greater.

There were no associations between immunoglobulin levels and CVD or cancer mortality, or deaths from external causes (of which half of the 63 deaths in this category were from suicide or homicide). However, for ‘other’ causes of death, mainly comprising infectious disease mortality, individuals with higher IgA and IgM levels had, respectively, a 4.7 and 3.5 times increased risk of dying. For IgG, those with levels one SD above the mean were 33 times more likely to have died from these other causes. Death from ‘other’ causes was also associated with greater alcohol consumption, higher cholesterol, non-white ethnicity, lower household income in midlife, being an ex-smoker, being unmarried and having a physical illness. These covariates account for the reduction in HR in the multiply adjusted models. Those with the greatest impact were being in the lowest income group, never being married or now divorced/separated, and being non-white, which all had HRs of 2.0 or greater.

Figure 2 shows quartiles of IgG against all-cause and ‘other’ cause mortality and would appear to indicate that the relationship reflects a threshold rather than a dose–response effect. As the ‘other’ causes category is quite a mixed category, we have conducted further multiply adjusted analyses for the larger disease groupings within this category. For deaths from infectious disease, comprising HIV and all other types of infections (ICD-9 codes: 001-033034.1-134136-139771.3, ICD-10: A00-B99) (N=19), IgA was not related to mortality, but IgG and IgM were significantly and positively associated with increased risk of death (p<0.001). For diseases of the digestive system (N=17), IgA, IgG and IgM predicted increased mortality risk (p≤0.001; data not shown). However, the HR sensitivity analyses in these instances are not interpretable due to the small group numbers. Finally, for deaths from liver disease including cirrhosis, of which there were 10, none of the immunoglobulin classes were statistically significant (data not shown).

Owing to the correlations between Ig titres (colinearity), we did not adjust the above analyses for the other Ig classes, thus making it difficult to identify the ‘independent’ effects of IgA, IgM, and IgG.

### Table 3
<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td>IgA (mg/L) logged</td>
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<tr>
<td><strong>Age-adjusted models</strong></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality (N=236)</td>
<td>2.40 (1.77 to 3.25)</td>
</tr>
<tr>
<td>Cardiovascular Disease (CVD) mortality (N=63)</td>
<td>1.73 (0.98 to 3.06)</td>
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<tr>
<td>Cancer mortality (N=47)</td>
<td>1.65 (0.85 to 3.18)</td>
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<tr>
<td>External mortality (N=63)</td>
<td>1.12 (0.62 to 2.00)</td>
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<tr>
<td>Other mortality (N=75)</td>
<td>7.68 (4.34 to 13.57)</td>
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<tr>
<td><strong>Multiply adjusted models</strong></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality (N=236)</td>
<td>2.00 (1.47 to 2.73)</td>
</tr>
<tr>
<td>CVD mortality (N=63)</td>
<td>1.47 (0.81 to 2.63)</td>
</tr>
<tr>
<td>Cancer mortality (N=47)</td>
<td>1.82 (0.91 to 3.63)</td>
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<tr>
<td>External mortality (N=63)</td>
<td>1.02 (0.58 to 1.81)</td>
</tr>
<tr>
<td>Other mortality (N=75)</td>
<td>4.65 (2.64 to 8.19)</td>
</tr>
</tbody>
</table>

*Adjusted for age, units of alcohol per week, Systolic blood pressure, cholesterol, body mass index, place of service, ethnicity, educational level, household income, smoking status, marital status and physical illness.

who had died in the first 7 years of follow-up (N=83). For all-cause mortality (N=153), the risk associated with high IgA levels remained, and was barely attenuated (HR=1.92, 95% CI 1.31 to 2.81). This was also the case for IgM (HR=1.60, 95% CI 1.14 to 2.24) and IgG, (HR=3.97, 95% CI 1.95 to 8.08). Similarly, for ‘other’ causes of death (N=52) the associations remained substantial; for IgA, HR=3.97, 95% CI 2.01 to 7.84, IgM, HR=3.52, 95% CI 2.08 to 5.96, and IgG, HR=19.29, 95% CI 7.02 to 53.02—(data not shown).

DISCUSSION

In the present study, higher levels of IgA, IgM, and IgG were associated with increased risk of mortality, and specifically mortality from ‘other’ causes, which largely includes infectious diseases and liver diseases. This remained the case after adjustment for a range of covariates associated, in this and other studies, with mortality. The fact that the associations were not only evident for early deaths, but for mortality that occurred later in follow-up suggests that this was not merely a case of occult (undiagnosed disease) being reflected in elevated immunoglobulin levels, and presaging an imminent death. Rather, high immunoglobulin levels are the results of activated immunoglobulin levels, and presaging an imminent case of occult (undiagnosed disease) being reactivated later in follow-up suggests that this was not merely a case of occult disease. Indeed, as we postulate above, it is probable that high IgA, IgM, and IgG levels result from nascent yet occult disease. Indeed, when the analyses for IgA and IgM are adjusted for levels of IgG, they are no longer significant (data not shown here), suggesting that their association with mortality perhaps only reflects their association with IgG. However, the colinearity between these measures means that it is difficult to separate out the individual immunoglobulin effects.

The present study has a number of limitations. First, in observational studies it is not possible to determine causality. It is likely that higher immunoglobulin levels are markers for some or several unmeasured factors related to mortality. Indeed, as we postulate above, it is probable that high IgA, IgM, and IgG levels result from nascent yet occult disease. However, the present findings survived adjustment for a pre-existing diagnosis of cancer, diabetes and heart disease, suggesting that other diseases are responsible for the mortality observed. Second, the sample was exclusively male so these findings cannot be readily generalised to women. However, given that men are more likely to have subnormal secretory IgA levels than women, it is possible that the relationship between Ig and mortality in women might be even stronger. Third, it is likely that there is also increased secretion of serum free light chains, as well as high overall Ig, which have been shown to positively relate to increased mortality. Finally, although this analytical sample is based on the recruitment of a random sample of surviving men, concerns about selection bias are nonetheless possible; that is, if the reported results differ markedly between persons included in the analyses and those not. There were no differences between the excluded and included participants according to age or social background, and only a slightly higher incidence of service experience in Vietnam as opposed to elsewhere between men in the analytical sample (55%) and the group not in the initial random sample of surviving men (51%, p<0.001). The fact that this marginal difference reached statistical significance can be ascribed to the large sample size.

In conclusion, higher immunoglobulin levels, particularly levels of IgG, were associated with all-cause mortality and with death from mainly infectious disease. As such, high serum immunoglobulin levels could be signifying nascent yet still occult infectious disease and, accordingly, provide a useful additional diagnostic and prognostic tool.
High levels of immunoglobulins can indicate disease, although they are protective against infection.

What this study adds

High levels of immunoglobulins are associated with total and ‘other’ cause (mainly infectious diseases) mortality, potentially indicating nascent disease.

Contributors ACP, DC and GDB developed the idea. ACP and DC conducted the analyses. ACP, DC, GDB and MTD contributed to writing the manuscript.

Competing interests None.

Patient consent Obtained.

Ethics approval Various relevant bodies, including the US Centers for Disease Control.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement These data are available with the kind permission of the Centers for Disease Control.

REFERENCES