Increased mortality of incident rheumatoid arthritis *versus* matched non-RA control subjects: a community-based long-term prospective cohort study

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Abstract

Objective

This study aimed to critically investigate all-cause and major-cause mortality of incident rheumatoid arthritis (RA) cases versus matched non-RA comparison (CN) subjects in a long-term prospective cohort.

Methods

Baseline 1974 cohort entry demographic and serum biomarker data on 54 incident RA patients and 216 matched CN subjects were related to their mortality from 1995 through 2015. Mortality of RA patients was also analysed by 3 categories of course responses to therapy assigned by the sole community rheumatologist in 1995 (19 good, 23 fair, and 12 limited). Cox proportional hazards regression models including baseline covariates were used to determine survival from all-causes, cardiovascular disease (CVD), respiratory-related, malignancies, and other causes of death (CODs).

Results

Total deaths occurred in 38 (70.4 percent) of 54 RA and 102 (47.7 percent) of 216 CN (p=0.003). Total mortality remained greater (p=0.011) in RA versus CN subjects after adjustment for baseline demographic covariates (HR= 1.66, 95% CI 1.12–2.46). Respiratory-related CODs were also greater (p=0.047) in RA versus CN (HR= 2.69, 95% CI 1.02–7.14) subjects. The RA patients' responses to therapy in 1995 significantly (p=0.004) predicted total mortality. Baseline serum immunological and steroid biomarkers independently predicted total, CVD, and other and unknown CODs.
Pre-clinical (1974) ranked biomarker z-score values (1 = lowest, 5 = highest) within matched sets of 1 RA and 4 CN study subjects independently associated with mortality from 1995 through 2015, for both total (CRP, p=0.028 and sIL-2Rα, p=0.030) and CVD (CRP, p=0.005 and sTNF-R1, p=0.003) deaths.

Conclusion

Total mortality and respiratory-related CODs were greater in incident RA versus CN subjects. The 35 RA cases who had fair or limited course responses to rheumatologist's therapy had greater mortality than their matched CN, whereas the 19 good RA responders had equivalent survival to CN subjects. The independent CRP and sTNF-R1 biomarker associations with CVD deaths were enhanced by a gradient of their dichotomous z-score values in survival models.

Key words

rheumatoid arthritis, mortality, cardiovascular disease, respiratory, disease course

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Introduction

Mortality of rheumatoid arthritis (RA) patients has been a long-standing research topic. Approximately 50% lower survival was reported in a cohort of RA patients from 1966 to 1974 in Saskatchewan, Canada than in population controls (1). Decreased survival was attributed to direct disease-related causes, like severity and infection. A 2001 population-based study in northern Norway (2) indicated a two-fold increased mortality in RA patients, but not significantly increased for cardiovascular disease (CVD) or cancer. A greater mortality was reported in RA patients from Finland than in the general population, including urogenital, gastrointestinal, respiratory and CVD as well as from infections and malignancies (3). A further report on the latter Finnish population (4) indicated that the increased mortality in RA patients occurred among those treated for more than 10 years with low-dose oral glucocorticoids (GCs).

A 2007 cohort study from the UK (5) found a greater mortality over 15 years of follow-up among RA patients (54%) than in same-sex siblings (28%) or patients with knee osteoarthritis (OA) (32%). The RA and OA patients had a greater relative frequency of ischaemic heart disease than the siblings (5). In a study of RA patients first diagnosed at Mayo Clinic between 1995 and 2000, mortality was greater than in the general population and was relatively constant at 2.4 (females) and 2.5 (males) per 100 person-years (6). In a follow-up study (7), the increased mortality of RA patients and widened gap from the general population was attributed to RA subjects who had positive rheumatoid factor. Their relative risk of death was greater for CVD (1.50, 95% CI 1.22, 1.83) and respiratory diseases (3.49, 95% CI 2.51, 4.72) (7). The rheumatoid factor (RF)-negative RA patients had nearly identical mortality to the control population (7). A 2010 population-based study of RA mortality from Denmark (8) revealed an increased standardised mortality ratio (SMR) from all-causes (1.5, 95% CI 1.2–1.9) and from CVD (1.7, 95% CI 1.3–2.6).

Among RA patients who had onset since 2000 from British Columbia (9), their all-cause mortality per 1000 person-years (18.3) was similar to the control population (17.9). Mortality from CVD causes was also similar in the RA (5.7) and control (5.9) populations (9). Among RA patients diagnosed between 2000 and 2007 at the Mayo Clinic (10), the 10-year overall percentage cardiovascular mortality was slightly lower (2.8±1.2) than in the control population (3.3±1.2). The recent 10-year CVD mortality of RA patients was decreased from the preceding 1990–99 incidence cohort (10). Interstitial lung disease contributed approximately 13% to the *excess* mortality in a recent Mayo Clinic series of RA patients compared to the general population (11). Although a lower mortality of RA patients since 2000 is suggested by the preceding reports (9, 10), 7-year mortality rates continued to be increased among early RA patients enrolled in a 2000-2004 cohort, particularly among those who were auto-antibody positive, RF (latex test) or anti-citrullinated protein antibody (ACPA) (12).

This long-term nested case-control prospective study investigates total and major cause-specific mortality of incident RA cases and matched non-RA subjects in relation to 1974 baseline pre-RA demographic and serum biomarker data and by 3 categories of course pattern responses to therapy of the RA cases, as assigned in 1995.

Subjects and methods

The RA Precursors Study (RAPS) at this Institution (13-15) and Operation CLUE I (16-18)

– Ethical approval

The RA Precursors Study (RAPS) was initiated at this institution in 1991 and has been approved by the University of Illinois College of Medicine at Peoria Institutional Review Board (Project Title: (85786-5) Rheumatoid Arthritis Precursors Study (RAPS) Database Project) (13-15). The RAPS study subjects are the community-based sample of 54 incident RA and 216 matched non-RA (CN) who had been derived from the 1974 CLUE I cohort (13-18). The baseline demographic data and total serum aliquots of the RAPS study subjects were donated by CLUE I, op-

erated by the Johns Hopkins - Bloomberg School of Public Health (16-18). The Institutional Review Board project number of the CLUE Cohort Study is 00005798, principal investigator, Dr. Kala Visvanathan, under the Johns Hopkins University – Bloomberg School of Public Health. All data on study subjects were anonymously collected and coded and do not require consent from individual patients.

The female CLUE I cohort consisted of 12,381 residents of Washington County, Maryland (13, 14) and the male cohort consisted of 8,680 residents (15). CLUE I participants have been followed longitudinally to the present. The baseline biomarkers in this study were assayed in reference laboratories under contract by RAPS (see assays below). The RA cases were ascertained by medical record review in the sole community rheumatologist's practice. Mortality outcome is determined by operation CLUE I from continuing collection of death notices and certificates (16-18). The primary purpose of Operation CLUE I is to study cancer biomarkers and risk factors (16-18). Female subjects who had known cancer diagnoses in follow-up to 1992 were excluded from the RAPS database of RA and CN subjects. Consequently, cancer-related causes of death (CODs) are diminished in the female RAPS database, but are not biased by pre-RA *vs*. CN categorisation. The malignancy exclusion criterion was not applied to the male RA cases identified in 1994 because of their smaller sample size.

In 1992, 36 women from the female cohort were identified by medical record review in the sole community rheumatologist's practice. All incident RA patients had developed clinical onset of disease following 1974 cohort entry (onsets after 3 to 18 (median 11) years), as previously described (14). In 1994, 18 males were identified as having developed clinical RA in the rheumatologist's practice following cohort entry (onsets after 3 to 20 (median 12) years) (15). The sole community rheumatologist diagnosed and confirmed all RA cases according to American College of Rheumatology (ACR) 1987 revised classification criteria (19). At cohort entry in 1974, all subsequently developed RA cases satisfied European League Against Rheumatism (EULAR) recommendations for incident pre-disease criteria (20). Four cohort CN subjects were matched to each pre-RA case on sex, race (all Caucasian), and usually within one year of age at entry, selected as the closest in chronological sequence of enrolment in the cohort to the pre-RA, analogous to another case-control study (21). No matched CN subject had a diagnosis of RA in the community rheumatologist's practice. At the start of the study in 1992, no RA or CN subject was known by operation CLUE to have deceased in regular mortality follow-up.

Serum assays and repeatability testing for the panel of baseline steroids and hormones

In female subjects, the panel of serum neuroendocrine biomarkers was assayed at a university referral laboratory in separate 1992 and 1994 batches on anonymously coded and stored (–70°C) 1974 baseline sera (13). In male study subjects, a more selective neuroendocrine panel was assayed in 1996 on coded sera at the same laboratory (15). The measurement criterion for acceptability of neuroendocrine assays was an intra-assay percentile coefficient of variation (% CV) less than 12%. In both sexes, assay priority was given to cortisol, dehydroepiandrosterone (DHEA), its sulfate (DHEAS), luteinising hormone (LH), and prolactin (PRL). In males, sera were also sufficient for complete assays of total testosterone (T) and estradiol (E2), two C19 androgenic steroid precursors (DHEA and androstenedione), cortisol, two C21 17-hydroxylated glucocorticoid (GC) precursors (17-hydroxypregnenolone and 17- hydroxyprogesterone), and two pituitary hormones (LH and PRL). In females, sera were sufficient for total assays of cortisol, DHEAS, and three pituitary hormones (FSH, LH and PRL), but missing in a minority of the other steroids (13, 15).

Assay methods for the baseline (1974) acute phase proteins and cytokine biomarker panel

Serum acute phase proteins and inflammatory cytokine biomarkers were assayed in the total female subjects in 1995 and in the males in 1996 on coded sera at national referral laboratories, as previously described (14, 15). Acute-phase serum amyloid A (ASAA) and C-reactive protein (hsCRP) were assayed by high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (mg/L) (14). The first 1992 batch of CRP assays in women was assayed at Northwestern University (14). The second batch of CRP assays, including ASAA, was performed at Boston University and CVs (±SE) were 2.7 (±2.0) and 6.6 (±2.8), respectively. The ASAA assays (n=200) could not be performed on the early batch females (n=70), as those sera had previously been exhausted. Serum cytokines (IL-1β, IL-6, and TNF-α), two cytokine receptors (sIL-2Rα and sTNF-R1), and a cytokine antagonist (IL-1ra) levels (pg/ml) were assayed using serum ELISA kits (R & D Systems Inc., Minneapolis, MN) (14). All cytokine assays were performed at Specialty Laboratories, Inc. (SLI), Santa Monica, CA. Serum assays were performed on the separate batches of earlier (1992)- and later (1994)- recruited women in April 1995 and in men in 1996, as previously described (14). Coefficients of variation of the cytokine assays were determined from repeat (2-7) assays of 9 standard sera, yielding the following overall mean (±SE) values: TNF-α (15.2±5.4); IL-1β (19.8±13.0); IL-1ra (11.2±3.9); IL-6 (11.3±4.1); sIL-2Rα (9.2±2.5), sTNF-R1 (8.0±2.7). All immunological assays in males were performed at the above indicated laboratories in 1996 (14). Isotope specific rheumatoid factor (RF) assays, IgA, IgG, and IgM were performed in TheraTest Laboratories, Chicago, IL and CVs (±SE) were between 16.0 (±2.1) and 20.2 (±1.6). Vascular endothelial growth factor (VEGF) was assayed in R&D Systems Laboratory, Minneapolis, MN on only second batch female samples (n=110). The standard sera for determining CV yielded 6.5 (±9.2) percent.

Assay values were log-transformed and standardised by Z-scores in females and males

All reported steroidal and hormonal (neuroendocrine) and immunological (NEI) assays were first transformed

by natural log to improve symmetry and distribution (13). Extreme outliers were identified in several variables, particularly the acute phase proteins, which are sensitive and highly reactive assays (22). Outliers were assigned by Winsorization technique to the respective lower and upper ranges in their frequency distributions (23). Subsequently, the log-transformed and Winsorized NEI values were converted to z-scores to standardise their respective variances within the 3 sets of subjects, *i.e.* early-batch females (n=70); late-batch females (n=110), and males (n=90). The z-score values were almost always distributed ±2 or ±3 standard deviations (SDs).

Categorisation of RA patients into 3 patterns of therapy responses (Rxresp) in 1995

In 1995, the RA patients were categorised into 3 therapy response (Rxresp) categories by the principal investigator (ATM), based upon the combination of: (1) concordance with the 1981 American College of Rheumatology (ACR) preliminary criteria for clinical remission (24); (2) community rheumatologist's retrospective assessment in 1995 of patients' therapeutic course patterns (1=good, 2=fair (same as moderate), 3=limited); (3) number of swollen joints (<10 *vs*. 10+), as well as (4) functional capacity dichotomised as full (I) *vs*. compromised (II, III or IV), as classified by the 1991 Revised American College of Rheumatology Criteria (25). A good Rxresp (coded as 1) was assigned to RA patients who qualified for ACR remission criteria (24). A good Rxresp code was also assigned to cases considered by the practicing rheumatologist as having a good course-wide therapy response plus having <10 swollen joints and full functional capacity (25). A good Rxresp was assigned in 19 cases. A fair (same as moderate) Rxresp (coded as 2) was assigned to patients considered by the rheumatologist as having a fair response and *either* <10 swollen joints *or* full functional capacity (n=23) (25). A limited Rxresp (coded as 3) was assigned to patients considered by the rheumatologist as having a fair or limited course response pattern plus 10 or more swollen joints and compromised functional capacity (n=12). The Rxresp categories (coded as 1, 2, or 3) were assigned in 1995, prior to initiating this survival status follow-up study and was independent of: therapy protocol; sex; entry age; onset age of RA, and duration of RA by 1995. To minimise any unrecognised or confounding host-related influences on differential mortality of the RA *vs*. CN subjects, the Rxresp code of each RA case was also assigned within each set to the 4 corresponding matched control subjects. That variable (Rxresp_matched CN covariate) was entered in the Cox regression models to identify any unexpected correlations with the three patterns of therapy responses.

Causes of death (CODs) coding

Underlying CODs were determined from death certificate records and examined independently by 2 investigators (ATM and AAR). The investigators' categorisation differed in only one of 140 underlying CODs and was settled by consensus. Underlying CODs were categorised by International Classification of Diseases (ICD) – 9th Revision for 1992–1998 and ICD – 10 for 1999–2015. Death certificates and underlying CODs were not available for 10 deceased CN subjects who had moved their residences out of Maryland (10 (9.8%) of 102 CN deaths). Those 10 CODs were included in the category of "other and undetermined causes".

Multiple imputation and statistical methods

Multiple imputation (MI) was utilised to enter a minority of randomly missing transformed biomarker z-scores into the 3 respective RA and CN data sets (26, 27) using SAS 9.2 Software (SAS Institute Inc., Cary, NC) (28). Markov chain Monte Carlo (MCMC) method was used to conduct MI with SAS (28). A single mean value (aggregated) for each of the NEI variables was derived from 10 imputed data sets, using the IBM SPSS 21.0.0.0 (IBM SPSS, 2012) programme AGGREGATE (29, 30). After the z-scores of the 3 subsets of subjects were imputed separately, they were merged into a total subject sample (n=270). Frequency distributions of the imputed biomarker values were always closely similar to the originally reported values for each variable.

Spearman's rank correlation (ϱ) was used to identify bivariate associations of mortality with baseline (1974) demographic and serum neuroendocrine immune (NEI) biomarker variables in the RA and CN subjects. Therapeutic response categories (good, fair, and limited) as assigned in 1995 were also correlated with mortality of RA cases and as assigned to matched CN subjects. Multivariable Cox proportional hazards models were used to determine survival from all-causes, CVD, respiratory-related, malignancies, and other or unknown CODs. Both the 1974 demographic and serum biomarker values as well as the 1995 therapy response categories of the RA cases and as assigned to the matched CN (rxresp_matchedCN) subjects were incorporated as covariates in survival models. To minimise subject selection factors and laboratory batch effects on assays, individual biomarker z-scores were also analysed non-parametrically as ranks within the separate matched sets of 1 RA and 4 CN (1=lowest, 5=highest). Non-parametric analysis (Mann-Whitney U-test) within matched sets was used to test differences (p<0.050) in z-score ranks of NEI baseline biomarkers between RA and CN subjects. The z-score differences between RA *vs*. CN were tested between total decedents *vs*. survivors, as well as a major COD subgroup (*e.g.* CVD) *vs*. the remaining subjects. Censoring was performed to exclude selected data subsets, *e.g.* in comparisons of males *vs*. females and major CODs.

In addition to continuous and ranked z-scores of individual serum biomarkers, negative *vs*. positive (neg_pos) dichotomies of z-score values were studied (all negative *vs*. 0 and positive z-scores). Pairs of dichotomous biomarkers were also combined and analysed, *e.g.* CRP with sIL-2Rα or CRP with sTNF-R1. In addition, a 3-category gradient of dichotomous (negative *vs*. positive) CRP and sTNF-R1 z-scores was created (0=neither positive, 1=either positive, 2=both positive). In this exploratory

Table I. Numbers of total and major causes of death (COD) in 54 incident rheumatoid arthritis (RA) and 216 matched non-RA cohort controls*.

Underlying major causes of death	RA (n=54) n (%)	CN (n=216) n (%)	Odds ratios (95% C.I.)	RA *vs*. CN *p*-values
Cardiovascular	12 (22.2)	38 (17.6)	1.34 (0.64-2.78)	0.435
Respiratory	8 (14.8)	11 (5.1)	3.24 (1.23-8.51)	0.017
Neoplasms	8 (14.8)	18 (8.3)	1.91 (0.78-4.67)	0.154
Other, unknown	10 (18.5)	35 (16.2)	1.18 (0.54-2.55)	0.683
Total deaths	**38 (70.4)**	**102 (47.7)**	**2.65 (1.40-5.05)**	**0.003**

*Deaths occurred between 1984 to 2015, and CODs were not obtained in 10 (4.6%) of CN, but none in RA.

study, a significance level of $p \leq 0.050$ was accepted without adjustment for multiple comparisons (31).

Results

Numbers of total and major causes of death (CODs) in the RA and CN subjects (Table I)

The 38 (70.4%) of 54 observed deaths in RA cases is significantly (p=0.003) greater than the 102 (47.2%) in 216 matched CN subjects (OR=2.65, 95% CI 1.40–5.05) (Table I). Cardiovascular disease (CVD) was the most frequent COD in both study groups, 12 (22.2%) in RA *vs*. 38 (17.6%) in CN (OR=1.34, 95% CI 0.64–2.78). Respiratory-related CODs was significantly (p=0.017) greater in RA, 8 (14.8%) than in CN, 11 (5.1%) subjects (OR=3.24, 95% CI 1.23–8.51). Respiratory-related CODs were particularly increased (p=0.004) in RA women, 6 (16.6%) of 36 than in CN women, 4 (2.8%) of 144 (OR=7.00, 95% CI 1.86–26.34). In males, the respiratory-related COD was similar (p=0.861) in the groups, 2 (11.1%) of 18 RA *vs*. 7 (9.7%) of 72 CN (OR=1.16, 95% CI 0.22–6.13). Excluding respiratory deaths, mortality was not quite (p=0.078) greater in RA (55.6%) *vs*. CN (42.1%) subjects (OR 1.72, 95% CI 0.94–3.13).

Greater RA mortality independent of demographic covariates (Table II, Fig. 1A-B)

The greater RA *vs*. CN mortality from all causes persisted (p=0.011) in Cox regression analysis, independently of the 1974 baseline covariates (Table II, Fig. 1A). Cohort entry age (p<0.001) and years of completed education (p=0.009) were the strongest predictors of total mortality. Neither sex nor the 7-scale cigarette smoking score predicted total mortality.

Mortality in each separate COD category was significantly predicted by entry age, as may be expected (Table II). The education covariate independently and negatively predicted mortality from all causes and malignancy-related CODs. Cigarette smoking was a positive predictor of respiratory-related CODs (Ta-

Table II. Cohort entry (1974) demographic variables as correlated with total and major causes of death categories*.

Entry covariates as correlated with mortality	All deaths *vs*. alive (140 *vs*. 130)	CVD *vs*. others (50 *vs*. 217)	Respiratory *vs*. others (19 *vs*. 250)	Cancer *vs*. others (26 *vs*. 243)†	Other COD *vs*. others (45 *vs*. 225)
Cigarettes smoked (7-scale):					
p-values	0.089	0.810	0.012	0.083	0.594
Exponent (β)	1.112	0.970	1.498	1.203	0.924
(95% CI's)	(0.984-1.256)	(0.753-1.248)	(1.081-1.887)	(0.976-1.482)	(0.690-1.237)
Sex (F=0, M=1):					
p-values	0.916	0.231	0.809	0.001†	0.122
Exponent (β)	1.021	0.639	1.139	4.843	0.513
(95% CI's)	(0.692-1.507)	(0.307-1.330)	(0.396-3.2723)	(1.934-12.129)	(0.220-1.195)
Cohort entry age (decade):					
p-values	<0.001	<0.001	<0.001	0.026	<0.001
Exponent (β)	2.629	2.587	3.169	1.845	2.829
(95% CI's)	(2.119-3.261)	(1.829-3.660)	(1.640-6.123)	(1.078-3.158)	(1.964-4.074)
Education completed (yrs):					
p-values	0.009	0.214	0.164	0.048	0.411
Exponent (β)	0.914	0.930	0.876	0.858	0.950
(95% CI's)	(0.854-0.978)	(0.829-1.043)	(0.727-1.056)	(0.737-0.999)	(0.841-1.073)
Study groups (CN=0, RA=1):					
p-values	0.011	0.228	0.047	0.178	0.370
Exponent (β)	1.663	1.520	2.692	1.836	1.474
(95% CI's)‡	(1.123-2.461)	(0.770-3.002)	(1.015-7.138)	(0.759-4.442)	(0.701-3.101)

*All p and exponent B (95% CIs) values were derived from Cox regression models with simultaneous entry of all demographic and study group variables in a block; †Female CN and RA subjects were excluded, if they were recognised in 1992 to have had a malignancy diagnosis which artificially influenced the association with sex; ‡The Exp(β)-(95% CIs) entries indicate significantly greater risk of death for RA *vs*. CN in the total and respiratory-related causes.

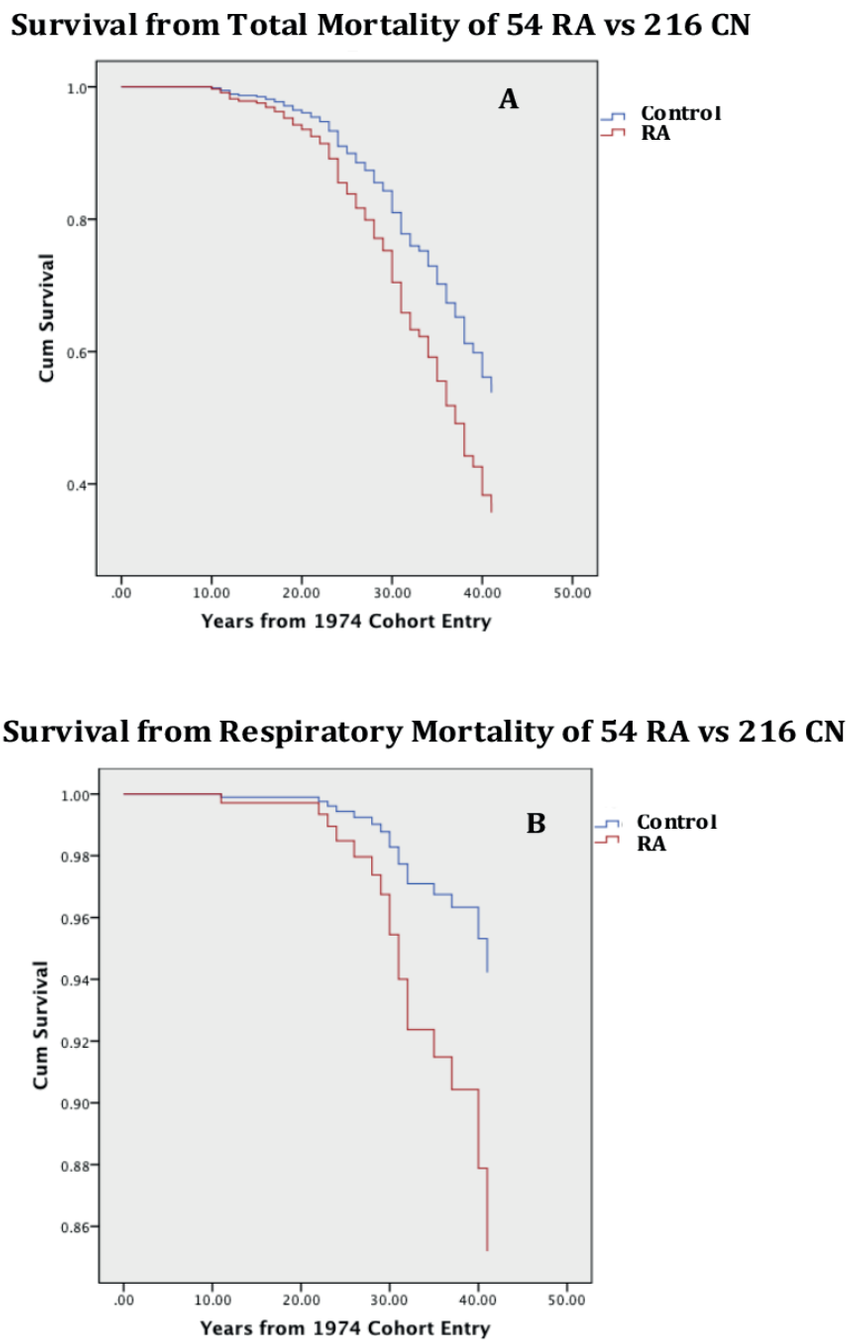

Fig. 1. Cox proportionate models of survival from total deaths (**A**) and from respiratory-related deaths (**B**) of RA *vs*. CN subjects followed through 2015, including covariates of decades of cohort entry age, years of education completed, sex (F=0, M=1), and history of cigarettes smoked (7-scale).

ble II). The RA *vs*. CN status (p=0.047) also predicted respiratory-related CODs, including the demographic covariates in the model (Table II, Fig. 1B).

Survival of RA by 3 categories of therapy responses, assigned in 1995 (Table III, Fig. 2)

The 3 categories of disease responses to therapy (Rxresp) assigned in 1995 significantly (p=0.012) associated with numbers of deaths in the 54 RA cases: 9 (47.4%) in 19 good response cases; 19 (82.6%) in 23 fair response cases, and 10 (83.3%) in 12 limited response cases (Table III, Fig. 2). The ages of RA cases (mean ± SE) in 1995 significantly (p=0.017) increased from good (60.0±2.5), to fair (65.3±2.1), and to limited (68.1±3.7) therapy response categories. The male to female ratio was greater (p=0.037) in good response cases (10 M : 9 F) than in the remaining 35 RA patients (8 M : 27 F). Each therapy response category of the RA cases was assigned to their matched CN to test for differences within and between categories. Total mortality did not differ (p=0.835) between RA *vs*. CN in the 19 sets of good RA responses and their matched CN, but was significantly greater in RA *vs*. CN for the 23 fair response (p=0.026) and the 12 limited response (p=0.017) sets in Cox regression models, including the demographic covariates (Table III, Fig. 2).

In the 54 RA cases, 9 (47.4%) of the 19 good response subjects deceased *vs*. 29 (82.9%) of the 35 remaining fair and limited response cases (p=0.012) (Table III). That mortality differential by therapy responses was significant (p=0.002) only in the 39 RF positive cases, 4 (36.4%) deceased of 11 assessed as good *vs*. 25 (89.3%) of the remainder 28 cases (Table III). In 54 RA, those 9 cases who had positive IgA or IgM RF at both 1974 cohort entry and 1995 clinical assessment had independently (p=0.010) greater mortality than either the 15 cases consistently sero-negative or 30 sero-positive only at clinical assessment, in Cox regression models including demographic covariates (data not shown). The dichotomous responses to RA therapy (good *vs*. fair/limited) independently (p=0.017) predicted mortality from the preceding RF subgroups (data not shown). In the 19 cases with good therapy responses, no significant difference occurred by sero-negative *vs*. sero-positive status (p=0.689) *vs*. the 35 with fair or limited responses (p=0.001), in Cox regression models including demographic covariates (data not shown).

Baseline (1974) pre-clinical biomarker predictors of all causes mortality (Table IV)

Two baseline serum biomarkers independently predicted total mortality in Cox proportionate survival models including baseline covariates and the CN *vs*. RA status: CRP (p=0.028) and sIL-2Rα (p=0.030) (Table IV). A significant (p<0.001) mortality trend oc-

Table III. Numbers and percentages of deaths in 3 categories of responses to therapy: good (n=19), fair (n=23), and limited (n=12) course patterns to 1995.

Deaths in RA study group (n=54), sex, age at RA onset, age in 1995, and duration (yrs) of RA in 1995	Good therapy response (n=19): Deaths - n (%)	Fair therapy response (n=23): Deaths - n (%)	Limited therapy response (n=12): Deaths - n (%)
Deaths (%) in RA categories	9 (47.4%)*	19 (82.6%)	10 (83.3%)
Total Females in categories	9 (47.4%)	19 (82.6%)	8 (66.7%)
Total Males in categories	10 (52.6%)†	4 (17.4%)	4 (33.3%)
Age at RA onset (mean ± SE)	52.2 ± 2.9	55.6 ± 2.3	59.0 ± 3.2
Age in 1995 (mean ± SE)	60.0 ± 2.5	65.3 ± 2.1	68.1 ± 3.7
Yrs of RA in 1995 (mean ± SE)	7.8 ± 1.2	9.7 ± 0.7	9.1 ± 1.2
RA *vs*. CN total CODs, Exp β (±SE), p‡	1.08 (0.52-2.23), 0.835	1.80 (1.07-3.01), 0.026	2.28 (1.16-4.48), 0.017

*p=0.012 (less deaths in good *vs*. other therapy responses); † p=0.037 (more males in good *vs*. other therapy responses); ‡p values of RA *vs*. CN total deaths were derived as the dependent outcome in each therapy response category by multivariate logistic regression of mortality outcomes of respective cases and their matched control subjects. Ages in 1995 differed (p=0.017) by the therapy responses.

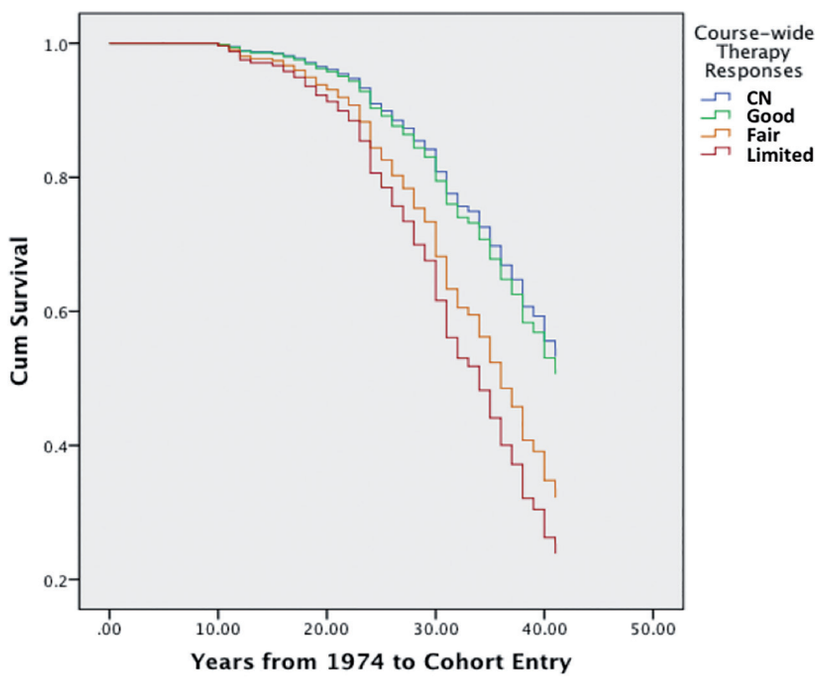

Fig. 2. Cox proportionate models of survival from total deaths of RA patients by rheumatologist's categorisation into 3 therapy response (Rxresp) course patterns, in 1995: good=1; fair=2, and limited=3, including covariates of decades of cohort entry age, years of education completed, sex (F=0, M=1), and history of cigarettes smoked (7-scale).

curred when the total 140 deaths (RA and CN) were analysed in a 3-scale gradient of the dichotomous (neg/pos) biomarker pairs: 25 (33.8%) deaths in 74 subjects who had both biomarker z-scores negative; 67 (51.9%) deaths in 129 who had either biomarker positive, and 48 (71.6%) deaths in 67 subjects who had both z-scores positive. Comparable trends occurred in the RA (54.5%, 65.2%, and 85.0%, respectively, p=0.159) and CN (30.2%, 49.1%, 66.0%, respectively, p<0.001).

Baseline (1974) pre-clinical biomarker predictors of CVD mortality (Table V, Fig. 3)

Two baseline serum biomarkers independently predicted CVD mortality in Cox proportionate survival models including baseline covariates and the CN *vs*. RA status: hsCRP (p=0.005) and sTNF-R1 (p=0.003) (Table V). The CRP and sTNF-R1 biomarkers were significant (p=0.016 and 0.016, respectively) predictors in the pre-RA, but not quite (p=0.066 and 0.074, respectively) in CN subjects. A significant CVD mortality gradient was observed between the reference survival of both biomarker z-scores negative and sTNF-R1 positive alone (p=0.017), CRP positive alone (p=0.024), and both z-scores positive (p=0.001) (Fig. 3). In a 3-gradient scale, CVD deaths occurred in 5 (5.8%) of 86 subjects who had both biomarker z-scores negative, 23 (20.9%) of 110 subjects who had either biomarker positive, and 22 (29.7%) of 74 subjects who had both positive (p<0.001). The 3-scale biomarker gradients of mortality for CN were 5.5%, 28.9%, and 25.9%, respectively (p=0.003) and for RA were 7.7%, 19.0%, and 43.8%, respectively (p=0.055).

Ranks of each of the baseline NEI z-scores were searched as independent predictors of the remaining CODs, *i.e*. respiratory-related, malignancies, and the remainder of other or unknown deaths with only one positive finding. Rank of testosterone was a significant (p=0.027) predictor of the 45 other and unknown CODs (Exp β 0.78 (0.64–0.97)), which was also significant (p=0.049) in the 38 women, but not in 7 men.

Discussion

Mortality in RA patients has been related to: positive serum rheumatoid factor (7); disease severity manifestations (32); glucocorticoid usage for more than 10 years (4); history of smoking, and older age (32). The latter mortality association is expected in the general population, as are influences from educational attainment or other socioeconomic-related factors (33) and sex (34). Some studies of RA patients followed after 2000 indicated an equivalent mortality to CN subjects (9, 10), although not found in another report (12).

In this study, the RA *vs*. CN status was a significant (p=0.011) predictor of total mortality after adjustment for demographic covariates (Table II) and addition of baseline serum immunologic biomarker covariates (Table IV). An unexpected strong (p<0.001) positive history of current cigarette smoking at cohort entry (Table III) was found in the 19 good response cases

Table IV. Baseline (1974) demographic and serum biomarker predictors for all deaths through 2015 in 270 total, 54 rheumatoid arthritis, and 216 matched cohort control subjects*.

1974 Demographic and biomarker predictors of mortality through 2015	Total Subjects (n=270)		Incident RA (n=54)		Cohort Control (n=216)	
	Exp β (±SE)	*p*	Exp β (±SE)	*p*	Exp β (±SE)	*p*
Cohort entry age (deciles)	**2.80 (2.26-2.47)**	**0.000**	**2.35 (1.58-3.50)**	**0.000**	**3.06 (2.38-3.94)**	**0.000**
Sex (F=0, M=1)	**1.17 (0.78-1.75)**	0.448	1.63 (0.70-3.79)	0.261	0.97 (0.59-1.58)	0.888
Cigarettes smoked (7-scale)	**1.10 (0.97-1.25)**	0.137	0.94 (0.74-1.21)	0.645	1.18 (1.02-1.38)	0.032
Education completed (yrs)	**0.94 (0.88-1.01)**	0.108	1.07 (0.89-1.29)	0.455	0.92 (0.85-0.99)	0.025
Subject groups (CN=0, RA=1)	**1.66 (1.12-2.43)**	0.011	N/A	N/A	N/A	N/A
Therapy responses_matched*	**1.37 (1.04-1.71)**	**0.022**	**1.65 (0.90-3.02)**	**0.108**	**1.21 (0.91-1.61)**	**0.198**
CRP (set rank)†	**1.16 (1.02-1.32)**	0.028	1,33 (0.99-1.80)	0.061	1.14 (0.97-1.34)	0.106
sIL-2Rα (set rank)‡	**1.15 (1.01-1.30)**	0.030	1.24 (0.94-1.64)	0.130	1.11 (0.95-1.29)	0.192

*All entry values reflect results when the rxresp_matched CN covariate is included in the models;
† Total subjects Expβ 1.14 (0.99-1.31), *p*=0.076 with Rank IgM in model and Expβ 1.11 (0.95-1.28), *p*=0.183 with Rank IgA in model;
‡ Total subjects Expβ 1.17 (1.01-1.35), *p*=0.034 with Rank IgM in model and Expβ 1.07 (0.91-1.25), *p*=0.412 withy Rank IgA in model.

Table V. Baseline (1974) demographic and serum biomarker predictors of CVD deaths through 2015: 50 occurred in total subjects, 12 in rheumatoid arthritis, and 38 in matched controls*.

1974 Demographic and biomaker predictors of CVD mortality through 2015	Total Subjects (n=50)		Incident RA (n=12)		Cohort Control (n=38)	
	Exp β (±SE)	*p*	Exp β (±SE)	*p*	Exp β (±SE)	*p*
Cohort entry age (deciles)	**3.07 (2.14-4.40)**	0.000	**4.70 (1.82-12.10)**	**0.001**	**2.29 (1.94-4.34)**	**0.000**
Sex (F=0, M=1)	**0.69 (0.33-1.47)**	0.340	0.67 (0.10-4.56)	0.686	0.66 (0.27-1.59)	0.350
Cigarettes smoked (7-scale)	**0.99 (0.77-1.26)**	0.916	0.94 (0.53-1.66)	0.827	1.04 (0.77-1.39)	0.803
Education completed (yrs)	**0.96 (0.85-1.08)**	0.450	1.12 (0.71-1.77)	0.630	0.94 (0.83-1.07)	0.334
Subject groups (CN=0, RA=1)	**1.66 (0.84-3.28)**	0.148	N/A	N/A	N/A	N/A
Therapy responses_matched*	**1.08 (0.70-1.66)**	**0.737**	**1.87 (0.50-6.96)**	**0.351**	**1.03 (0.63-1.71)**	**0.896**
CRP (set rank)	**1.39 (1.11-1.75)**	0.005	2.33 (1.17-4.62)	0.016	1.28 (0.98-1.65)	0.066
sTNF-R1 (set rank)	**1.39 (1.12-1.72)**	0.003	2.36 (1.18-4.73)	0.016	1.25 (0.98-1.59)	0.074

*All entry values reflect results when the rxresp_matched CN covariate is included in the models.

(n=18, 94.7 percent) than in their 76 matched CN subjects (n=29, 38.2%) and also greater ($p<0.001$) than in the remaining 35 RA cases who had either fair or limited therapy responses (n=16, 45.7%). Methotrexate therapy was used less frequently ($p=0.045$) in the good (n=6, 31.6%) response cases than in the remaining 35 RA patients (n=22, 62.9%, data not shown). However, administered weekly oral dosages were comparable (11.3, 13.0, and 12.5 mg, respectively, data not shown). This study did not attempt to interpret effects of drug administration on therapy response, since both variables were determined concurrently in 1995. In this RA cohort, the mean duration of disease was 8 years for the 19 good response patients and 9 years for the 35 remaining cases (Table IV).

Effects of therapeutic interventions on survival in RA, especially with respect to methotrexate (MTX), have been investigated in advanced, long-term patients (35), and in early, incident inception cases (36). Such analyses are difficult to interpret due to multiple factors influencing therapeutic choice, varied response to therapy and determinants of mortality outcome, all contributing to confounding by indication (37-39). In one study of definite RA patients with mean disease duration of 8.5 years (35), a favourable 1-year baseline response to MTX (≥20% improvement) in 165 patients was associated with subsequently improved 10-year survival (circa 75%) *vs*. 50 patients who had less than 20% improvement (circa 35%). However, no additional survival difference occurred in subsequent 8-year follow-up (35). In an early inception RA cohort, MTX was used more frequently in survivors over a median (range) duration of 4 (1-9) years follow-up than cases who died of either CVD or other CODs (36). The reverse relation was found for RA cases who had been treated with glucocorticoids, inferring that MTX is associated with lower CV mortality, opposite to use of glucocorticoids (36).

The RA *vs*. CN status was also a significant ($p=0.047$) predictor of respiratory-related CODs in the presence of baseline demographic covariates. Degree of cigarettes smoked was a predictor of mortality in the CN ($p=0.035$), but not in the RA subgroup (Table IV). Respiratory-related CODs were greater ($p=0.011$) among RA patients who had fair or limited therapy responses than their matched CN subjects (HR=2.02, 95% CI=1.17–3.48).

In the current study, age, but not sex, was a significant predictor of mortality in the total, RA and CN subgroups in the presence of other demographic and biomarker variables in the models (Table IV). A concept has been raised that accelerated constitutional aging may contribute to excess mortality in seropositive RA patients (40). Although RA and CN subjects were matched on

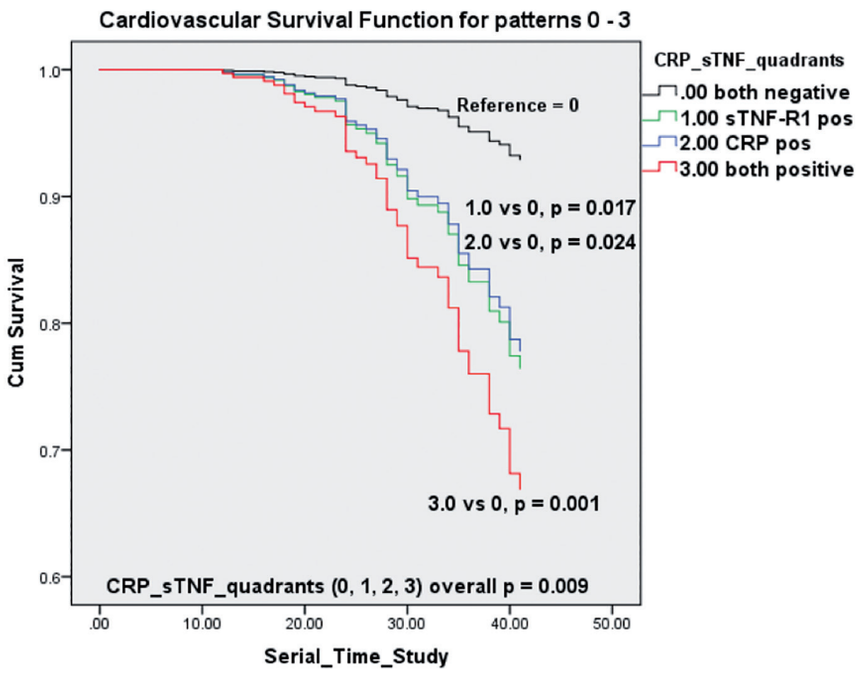

Fig. 3. Cox proportionate models of survival from CVD deaths by 4 gradients of 1974 baseline serum CRP and sTNF-R1 dichotomous z-scores (neg *vs*. pos), as shown in legend (0=both negative, 1= sTNF-R1 positive, 2 = CRP positive, 3 = both positive), including baseline demographic covariates as well as RA *vs*. CN status.

cohort entry age in this study, various other age-related factors were explored as possible contributors to total or major CODs. Earlier *vs*. later age at RA onset, duration of RA, and patient's age in 1995 were analysed (Table III), but none was a significant independent mortality predictor (data not shown). Androgenic-anabolic steroids have also been inferred to be a correlate of aging (41), but pre-clinical serum testosterone z-scores were the only neuroendocrine steroid to associate with lower mortality from other and unknown CODs (Exp β 0.79 (0.64–0.97), p=0.027). That factor was also a predictor of fewer all-cause mortality (p=0.028), but not independent of serum CRP and sIL-2Rα.

In a recent long-term Nurses' Health Study (NHS) (42), respiratory cause mortality was increased in women who developed incident RA, compared to women without this disease (HR, 2.06; 95% CI, 1.51–2.80). The greater respiratory disease mortality was observed only in RF-positive cases than the non-RA women (HR, 2.67; 95% CI, 1.89–3.77) (42). That NHS study (42) also found increased CVD mortality in the RA *vs*. non-RA women (HR, 1.45; 95% CI=1.14–1.83). A recent longitudinal observational study of male US veterans with RA found a 3-fold increased risk of respiratory-related death in cases compared to the general population expectation (32). We also found a significantly increased (p=0.017) respiratory-related mortality in total RA *vs*. CN subjects (OR=3.24, 95% CI=1.23–8.51) (Table I), particularly in women (OR=7.00, 95% CI=1.86–26.34). The greater respiratory mortality persisted after all demographic (p=0.047, Table II) as well as biomarker (p=0.034, data not shown) covariates were included in the Cox regression models. Degree of cigarette smoking (0–6 scale) was found to significantly predict respiratory-related mortality *vs*. all other subjects (Exp β 1.50 (1.08–1.89), p=0.012) (Table II).

Greater mortality has been reported in RA patients having positive rather than negative rheumatoid factor (7, 12, 43) and positive anti-CCP antibodies (44). This study only had available 1974 baseline isotype IgA and IgM RF titers in men and women, as well as the presence or absence of positive IgM RF titers in 1995. Neither of the baseline isotype RF biomarkers independently predicted mortality in the Cox regression models. However, when the RF variables were added to the models, including the ranked CRP and sIL-2Rα biomarkers, the RF variables diminished the respective ability of the biomarkers to predict mortality (Table IV, footnotes). The technical possibility that baseline IgA or IgM RF titers could have confounded other ELISA assay values (45) was also assessed. Each potential biomarker was analysed as the dependent outcome variable in linear regression models which included IgA RF and IgM RF separately as independent covariates, besides age, sex, and the CN-RA study group variables. Serum CRP levels were weakly predicted by IgA RF (β=0.197, p=0.040), but not by IgM RF (β=0.047, p=0.485) levels. Serum sIL-2Rα levels correlated strongly with IgA RF (β=0.325, p<0.001), but not with IgM RF (β=0.103, p=0.142) levels (Table IV, footnote). The sIL-2Rα biomarker remained a low predictor of total mortality (Exp β 1.17 (1.01–1.35), p=0.034) in a Cox regression model, including serum IgM RF levels (Table IV, footnote).

Measurement of 1974 baseline and pre-clinical serum biomarkers on study subjects is a distinctive aspect of this mortality study. The significant prediction of CVD mortality in total and CN subjects by ranked values of an inflammatory biomarker (CRP) and a cytokine receptor (sTNF-R1), either alone or in combination, is a provocative, but currently preliminary finding. On a 3-scale gradient of the 2 biomarkers z-scores: 0=neither positive; 1=either positive, and 2=both positive, percentile mortality incrementally increased in total (33.7, 56.4, and 66.2), CN (28.6, 54.1, and 60.3), and RA (61.5, 64.0, and 87.5) subjects, respectively. Analogously, mortality from CVD was strongly predicted by a combination of dichotomous z-scores (neg/pos) of CRP and sTNF-R1 (Fig. 3). The respective incremental trends of percentile mortality were analogous in total (5.8, 20.9, and 29.7), CN (5.5, 28.9, and 25.9), and RA (7.7, 19.0, and 43.8) subjects. Such biomarker predictive trends in RA patients (46) and CN subjects (47) are consistent with concepts of inflammatory pathogenesis of CVD, which deserves further investigation.

The predictors of total and CVD mortality in all subjects were slightly stronger in the 54 RA *vs*. 216 CN subgroups alone, but with overlapping confidence intervals. The ranked CRP and sTNF-R1 biomarkers predicted CVD mortality in the RA (p=0.016 and p=0.016, respectively), but not in the CN (p=0.060 and p=0.074, respectively) subjects. The relevance of biomarker predictors of mortality in this study is qualified, due to the many variables monitored.

A limitation of this observational study is the modest sample sizes of incident RA cases and matched CN subjects as well as the single-time assessment in 1974 of the baseline pre-clinical demographic and biomarker variables. In a future survival study, assays of serum biomarkers before and after clinical onset of disease would be advantageous. Categorisation of RA therapy responses was performed only at a single time, in 1995. Multiple assessments during the course of follow-up of disease activity or severity, as related to administered therapy, would have enhanced this study. Ideally, a cumulative impact of disease would be desirable to determine its impact on survival, independent of other demographic and biomarker variables. Other reservations of our findings include: (1) reduced generalisability of observations from a Caucasian, smaller community population; (2) inability to categorise course patterns of RA patients on biological drug therapy; (3) exclusion of most female (RA and CN) subjects who had known cancer-related diagnoses in 1992; (4) a limited number (4.6%) of CN subjects with unknown CODs due to having moved out of state; (5) an increased risk of Type 1 error for interpreting the biomarker analyses due to many NEI variables which were analysed as total and CVD mortality predictors, and (6) potential bias due to unobserved confounders in this observational study.

In addition, this detailed mortality analysis uncovered 12 (5.6%) of 216 CN subjects who had actually deceased prior to 1995 (CVD = 4; respiratory = 1; neoplasms=1; other=6). Exclusion of those 12 CN subjects strengthens the greater RA *vs*. CN total (p=0.0003) and respiratory-related (p=0.011) mortality, and does not yield additional significance in the remaining CODs. This report does not include the available data on corticosteroid nor methotrexate use in 1995, but no difference was observed in mean dosages of these medications within the three categories of responses to therapy. Therapeutic modalities after 1995 were not available in this study. Possibly, the more recently available anti-TNF-α inhibitors may have been used in this RA case population and may have affected mortality.

Strengths of this nested case-control study design (or a case-control in a cohort study) are its advantages for identification of biologic precursors of disease (48). In particular, the study includes: 1) a defined community-based population sample; 2) assays of 1974 baseline pre-clinical biomarker levels in RA and CN subjects; 3) the sole community rheumatologist's assessment in 1995 of RA patients' overall course responses to therapies, and 4) long-term mortality follow-up of study subjects through 2015. The positive findings in this study support the reported significant excess of total (4, 7, 32) and respiratory-related (11, 32, 42) mortality in RA *vs*. CN subjects, independent of the baseline demographic and biomarker covariates in the multivariable models. The rheumatologist's assessment of incident RA patients' therapy responses in 1995 also supports the reported increased mortality hazard of greater disease severity manifestations (32).

Conclusions

This long-term, community-based cohort study has confirmed an increased total and respiratory-related mortality of incident RA patients, with clinical onsets between 1977 and 1994, than matched CN subjects, independent of demographic and biomarker covariates. The increased total and respiratory-related mortality was observed only in those cases who had less than a good pattern of therapeutic response to the rheumatologist's management. A novel finding of this prospective study is that baseline dichotomous CRP (neg/pos) in combination with dichotomous sIL-2Rα predicted mortality in total subjects and that CRP combined with sTNF-R1 (neg/pos) z-scores predicted CVD mortality in total and incident RA subjects. The validity of current results deserves to be tested in independent and larger sample population studies.

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