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The influence of pre-existing diabetes mellitus on the host immune response and outcome of pneumonia: analysis of two multicenter cohort studies

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Abstract

Objective—Although diabetes mellitus is implicated in susceptibility to infection, the association of diabetes with the subsequent course and outcome is unclear.

Design and setting—Retrospective analysis of two multicenter cohorts. We determined the association of pre-existing diabetes on the host immune response, acute organ function, and mortality in patients hospitalized with community-acquired pneumonia (CAP) in the GenIMS study (n=1895) and on mortality following either CAP or non-infectious hospitalizations in the population-based cohort study, Health ABC (n=1639).

Measurements—Mortality rate within first year, risk of organ dysfunction, and immune responses, including circulating inflammatory (tumor necrosis factor, interleukin-6, interleukin-10), coagulation (Factor IX, thrombin-antithrombin complexes, antithrombin), fibrinolysis (plasminogen-activator inhibitor-1, and D-dimer), and cell-surface markers (CD120a, CD120b, HLA-DR, TLR-2 and TLR-4).

Results—In GenIMS, diabetes increased mortality rate within first year after CAP (unadjusted hazard ratio [HR]=1.41, 95% confidence interval [CI]=1.12–1.76, p=0.002), even after adjusting

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for pre-existing cardiovascular and renal disease (adjusted HR=1.3, CI=1.03–1.65, p=0.02). In Health ABC, diabetes increased mortality rate within first year following CAP hospitalization, but not after hospitalization for non-infectious illnesses (significant interaction for diabetes and reason for hospitalization [p=0.04]; HR for diabetes on mortality over first year after CAP 1.87, CI=0.76–4.6, p=0.16 and after non-infectious hospitalization=1.16, CI=0.8–1.6, p=0.37). In GenIMS, immediate immune response was similar, as evidenced by similar circulating immune marker levels in the emergency department and during the first week. Those with diabetes had higher risk of acute kidney injury during hospitalization (39.3% vs. 31.7%, p=0.005) and they were more likely to die due to cardiovascular and kidney disease (34.4% vs. 26.8% and 10.4% vs. 4.5%, p=0.03).

Conclusions—Pre-existing diabetes was associated with a higher risk of death following CAP. The mechanism is not due to an altered immune response, at least as measured by a broad panel of circulating and cell surface markers, but may be due to worsening of pre-existing cardiovascular and kidney disease.

It is a long-standing medical axiom that diabetes mellitus is a risk factor for infection.[1,2] However, once infection occurs, the effect of diabetes on mortality is less clear. Some studies suggest diabetes is associated with higher mortality after an infection, but others show no association.[3–7] Prior studies that showed the association between diabetes and higher mortality may be confounded by higher prevalence of pre-existing chronic conditions, such as chronic kidney disease (CKD) or cardiovascular disease.[8–10]

If diabetes indeed is associated with higher mortality after infection, underlying mechanisms are unknown. Several mechanisms could explain these survival differences. For instance, animal and human models of infection suggest that immune abnormalities in diabetes, such as higher pro-inflammatory,[11,12] pro-coagulant, and anti-fibrinolytic activity,[13,14] and higher expression of pathogen recognition cell-surface receptors,[15,16] could worsen during acute illness and increase mortality. An alternate mechanism for higher mortality among diabetics is increased risk of acute organ dysfunction due to higher chronic disease burden.

We therefore examined two multicenter observational cohort studies to understand the effect of diabetes on the host immune response and outcomes of pneumonia. We analyzed a cohort of patients with community-acquired pneumonia (CAP) enrolled in the Genetic and Inflammatory Markers of Sepsis (GenIMS) study, and the subgroup of the population-based cohort, Health, Aging, and Body Composition (Health ABC) study, who required hospitalization. In both cohorts, we tested the hypothesis that pre-existing diabetes is associated with increased mortality within first year, independent of pre-existing chronic diseases. We then determined whether higher mortality is attributable to CAP per se by comparing survival differences over the first year in Health ABC between CAP and non-infectious hospitalizations. Finally, in GenIMS we tested the hypothesis that survival differences between those with and without diabetes were due to differences in immune response and a higher risk of acute organ dysfunction during infection.

METHODS

Subjects and design

We analyzed subjects enrolled in the GenIMS cohort to assess differences in mortality, organ dysfunction, and immune response. GenIMS is a prospective multicenter observational cohort of subjects with CAP enrolled in emergency departments (ED) of 28 academic and community hospitals in four US regions, including southwestern Pennsylvania, Connecticut, southern Michigan, and western Tennessee. Eligibility criteria are shown in Table 1. Of the 2320 subjects enrolled, we excluded 288 patients because they

were discharged from the ED and an additional 137 patients because the clinical team ruled out CAP during the first three days of hospitalization, thus restricting the analysis to the remaining 1895 subjects.

To assess whether higher mortality is attributable to CAP, we analyzed subjects enrolled in the Health ABC study, a population-based observational cohort of 70–79 year old well-functioning participants. Health ABC participants were enrolled from the same geographic regions as GenIMS, including southwestern Pennsylvania (Pittsburgh) and western Tennessee (Memphis). Of the 3075 enrolled, we analyzed 1645 (53.5%) subjects who were hospitalized at least once during the first five years of follow-up. We excluded an additional six subjects whose diabetes status was not known, restricting the analysis to the remaining 1639 subjects. Methods to ascertain hospitalization have been described previously.[17] Briefly, we assessed outcomes after the first major hospitalization following enrollment, comparing mortality rate within first year among those initially hospitalized for CAP and non-infectious illnesses (see Appendix). For each cohort, the Institutional Review Boards at each site approved the study. Informed consent was obtained from participants or next of kin for GenIMS and from participants for Health ABC.

Outcome and clinical variables

For GenIMS and Health ABC cohorts, the primary outcome variable was all-cause mortality rate within first year (Table 1). We examined several additional secondary outcome variables in GenIMS. We examined cause-specific mortality using National Death Index codes. The validity of this method has been described previously.[18,19] To compare risk of developing acute organ dysfunction during hospitalization, we compared risk of severe sepsis (infection plus organ dysfunction) using Consensus criteria,[20] and the risk of individual organ dysfunction for six organ systems. For acute kidney injury, we used the RIFLE criteria.[21] The RIFLE criteria classifies acute kidney injury into three categories of severity using changes in serum creatinine and urine output (Risk, Injury, and Failure corresponding to mild, moderate, and severe kidney injury, respectively). Finally, we used measures of severity of illness at hospital presentation, including APACHE III and the Pneumonia Severity Index (PSI).[22,23] Details of methods to assess comorbid conditions in both cohorts, including diabetes, are included in Table 1. Microbiologic characteristics were available only in GenIMS and assessed using blood and sputum cultures (see Online Repository).

Laboratory procedures

We assessed differences in the immune responses to infection in GenIMS during hospitalization between those with and without diabetes by comparing changes in circulating concentrations of biomarkers within inflammatory (tumor necrosis factor-α [TNF], interleukin [IL]-6, IL-10), coagulation (Factor IX, thrombin-antithrombin complexes [TAT], antithrombin), and fibrinolysis (plasminogen activator inhibitor [PAI]-1, and D-dimer) systems and expression of cell surface markers (CD120a and CD120b [signaling receptors for TNF], HLA-DR, and Toll-like receptor [TLR]2 and TLR4) on presentation to ED and over the first week. Details of sample collection and processing have been described previously.[24]

We used automated chemiluminescent immunoassay analyzer (IMMULITE, Diagnostic Products Corp., Los Angeles, CA, USA) to analyze TNF, IL-6, and IL-10. We analyzed coagulation and fibrinolysis markers in a random subset of 734 subjects by a commercial laboratory (Esoterix, Agoura Hills, CA, USA). Specific methods and kits used were: D-dimer, latex immunoassay (Diagnostica Stago, Parsippany, NJ, USA); PAI-1, bio immunoassay (Biopool Chromolize, Biopool International, Ventura, CA, USA); AT,

chromogenic (BioMerieux, Rhône-Alpes, France); Factor IX, clot (BioMerieux); and TAT, ELISA (Behring, King of Prussia, PA, USA).

We analyzed cell-surface markers on ED presentation and on third and seventh day. We analyzed cell-surface markers in a subset of 624 subjects enrolled in hospitals located within 60 miles of the University of Pittsburgh because samples for cell-surface markers have to be analyzed within 48 hours. We obtained fluorochrome- or biotin-conjugated antibodies from eBioScience (San Diego, CA, USA) for TLR2, TLR4, and HLA-DR and from Invitrogen (Carlsbad, CA, USA) for CD120a and CD120b. We incubated these antibodies with whole blood, lysed the red blood cells, and then washed, fixed, and stored the remaining cells at −4 °C. We acquired cell-surface marker data within 48 hours of fixation on a BD FACSVantage SE flow cytometer (San Jose, CA, USA) and used BD CellQuest software. Additional details of the assays for all markers are included in the Online Repository.

Statistical analyses

We conducted univariate comparisons of clinical characteristics for subjects with and without diabetes using chi-square and student's t-test and their non-parametric counterparts when necessary in both cohorts. We compared mortality rate within first year by constructing Kaplan-Meier survival curves for subjects with and without diabetes and used the Cox proportional hazards model. For the proportional hazards model, we confirmed that the hazard ratios (HR) were similar over different intervals. We constructed serial models, including unadjusted, adjusted for demographic characteristics (age, sex, and race), and adjusted for demographic characteristics and chronic diseases associated with diabetes, such as cardiovascular disease and CKD. To assess if mortality differences were attributable to CAP, we compared mortality in those hospitalized initially for CAP and other non-infectious illnesses in Health ABC. We tested for an interaction between diabetes and the reason for initial hospitalization on mortality rate within first year.

We assessed mechanisms of increased mortality in GenIMS. We compared risk of severe sepsis and individual organ dysfunction using logistic regression analyses. We compared differences in immune response to infection, comparing biomarkers and cell-surface markers in subjects with and without diabetes at ED presentation and during the first week of hospital stay. We used regression analysis with mixed models to account for correlation of these markers over time. We also compared inflammatory and coagulation biomarkers at hospital discharge in the subset that was discharged alive and appeared to have recovered clinically to compare resolution of immune response. We have previously shown that those with unresolved immune response, as evidenced by higher concentrations of biomarkers at hospital discharge, had higher mortality over first year after hospital discharge.[25] To account for biomarkers that were truncated because they were below detection thresholds, we used Tobit models to compare biomarker concentrations.[26] Due to the large number of comparisons for the cell surface markers, we reported p-values adjusted for false discovery rate at 0.05.[27]

RESULTS

Baseline characteristics of the GenIMS cohort

Table 2 shows pre-hospitalization characteristics for 1895 subjects enrolled in GenIMS. The average age of the cohort was 67 years and two-thirds had a history of smoking. Respiratory disease was the most common chronic disease and occurred in approximately a third of the cohort, and cardiovascular disease occurred in a fourth of the cohort. Diabetes occurred in 384 (20.3%) subjects. The prevalence of other chronic diseases, such as CKD, cancer, and HIV, was low (<5%).

Subjects with diabetes, on average, were three years older than subjects without diabetes, but results did not reach statistical significance. Those with diabetes were more likely to have CKD (10.4% vs. 3.4%, p<0.0001) and cardiovascular disease (42.9% vs. 21.3%, p<0.0001). HIV was more prevalent among those without diabetes (2.4% vs. 0.3%, p=0.02) and only 1 subject with diabetes had HIV. No differences were seen in the prevalence of smoking, cancer, and respiratory disease between the two groups.

Blood or sputum cultures to determine microbiologic etiology were obtained in 1606 (84.7%) subjects within 48 hours of ED presentation. An etiologic agent was identified in 186 (11.5%) subjects and this frequency was similar among those with and without diabetes (12.6% vs. 11.3%, p=0.5). Among subjects in whom an etiologic agent was identified, Gram-negative organisms were more common among those with diabetes, Gram-positive organisms were more common among those without, and no differences were seen in the frequency of mixed or anaerobic organisms (7.5% vs. 3.1%, 4.2% vs. 7.5%, 1% vs. 0.7% for Gram-negative, Gram-positive, and mixed or anaerobic organisms, respectively, p=0.001).

Diabetes was associated with higher acute organ dysfunction in GenIMS

At ED presentation, subjects with diabetes had greater illness severity, as evidenced by higher PSI scores (111.1 vs. 96.9, p<0.0001), higher frequency of subjects with diabetes in PSI Classes IV and V (68.7% vs. 53.6%, p<0.0001), and higher APACHE III scores (59.5 vs. 55.2, p<0.0001). When points for the physiology components of the APACHE III score were compared, those with diabetes had a higher score compared to those without diabetes (42.6 vs. 39.8, p<0.0001).

Subjects with diabetes had higher risk of severe sepsis compared to those without diabetes (34.6% vs. 29.7%, OR=1.25, 95% confidence intervals [CI]=0.99–1.58), but results were not statistically significant (p=0.06). This association was not statistically significant when adjusted for age, sex, race, and pre-existing cardiovascular disease and CKD (OR=1.23, 95% CI=0.96–1.59, p=0.09).

Diabetes increased the risk of acute kidney injury (30.2% vs. 22.9% for subjects with and without diabetes, p=0.007) and this association remained significant when adjusted for age, sex, and race (p=0.02). The association persisted when subjects with CKD were excluded (29.3% vs. 23.6%, p=0.05). The higher risk of acute kidney injury was mainly due to increased risk of moderate (RIFLE-I) kidney injury (7.6% vs. 3.6%), and only small differences were observed in the risk of mild (RIFLE-R) (10.9% vs. 9.6 %) and severe kidney injury (RIFLE-F) (11.6% vs. 9.6%). No differences were observed in the risk of respiratory, neurologic, cardiovascular, coagulation, and liver dysfunction (Table 3). Furthermore, those with and without diabetes were equally likely to require mechanical ventilation (6.8% vs. 7%, p=0.87) and intensive care unit (ICU) admission (14.1% vs. 16.5%, p=0.25) and only a small difference was seen in the median length of hospital stay (7.8 vs. 7.4 days, p=0.02).

Diabetes was associated with higher mortality in GenIMS

Subjects with diabetes had higher mortality rates over first year (unadjusted hazard ratio [HR] was 1.41, CI=1.12–1.76, p=0.002, Figure 1 and Table 4). The HR remained unchanged and the association remained statistically significant when adjusted for demographics (HR=1.32, CI=1.05–1.66, p=0.01) and additionally adjusting for higher burden of cardiovascular disease and CKD (HR=1.3, CI=1.03–1.65, p=0.02).

The mortality at 1-year in individuals who developed acute kidney injury was higher compared to those who did not develop it, in both subjects with and without diabetes, but results did not reach statistical significance among subjects with diabetes (30.7% vs. 21.4%,

p=0.08 and 28.7% vs. 14.3%, p<0.0001 in subjects with and without acute kidney injury stratified by presence and absence of diabetes).

Causes of death were different in those with and without diabetes (p=0.03). Deaths due to cardiovascular disease and kidney disease were higher among those with diabetes (34.4% vs. 26.8% and 10.4% vs. 4.5% for cardiovascular and kidney disease). Deaths due to cancer were lower among those with diabetes (12.5% vs. 26.5%), but no differences were observed in deaths due to infection, chronic respiratory disease, and other causes (15.6% vs. 15%, 16.7% vs. 14.7%, and 10.4% vs. 12.5% among those with and without diabetes for infection, chronic respiratory disease, and other causes, respectively).

Diabetes was associated with higher mortality after pneumonia compared to non-infectious illnesses in Health ABC

The clinical characteristics of subjects in Health ABC at enrollment are shown in Table 2. The average age was 73 years and approximately half were whites and females. The prevalence of diabetes was similar in Health ABC and GenIMS (n=299, 18.2% and n=384, 20.3% in Health ABC and GenIMS cohorts). Compared to GenIMS, the prevalence of respiratory disease was lower in Health ABC (24.2% vs. 37.9%), whereas CKD was more common in Health ABC (24.5% vs. 9.8%). Similar to GenIMS, subjects with diabetes enrolled in Health ABC had higher prevalence of CKD (34.1% vs. 22.4%, p<0.0001) and cardiovascular disease (35.9% vs. 25.9%, p=0.03).

An interaction was observed between diabetes and the reason for the initial hospitalization on mortality within the first year. In the unadjusted model, pre-existing diabetes was associated with higher risk of death after hospitalization for CAP compared to those hospitalized for non-infectious illnesses (HR=1.87, CI=0.76–4.6 and 1.16, CI=0.8–1.6 for those hospitalized for CAP and non-infectious illnesses, interaction p=0.04, [Table 4]). This interaction persisted in models adjusted for demographics (HR=1.82, CI=0.72–4.62 and 1.08, CI=0.76–1.52 for those hospitalized for CAP and non-infectious illnesses, interaction p=0.04) and additionally adjusted for pre-existing cardiovascular disease and CKD (HR=1.8, CI=0.66–4.92 and 1.06, CI=0.75–1.51 for those hospitalized for CAP and non-infectious illnesses, interaction p=0.09). Forty-one participants who were hospitalized initially for non-infectious illnesses were subsequently hospitalized for infections. Excluding these subjects did not alter the results and an interaction between diabetes, reason for hospitalization, and mortality within the first year persisted (unadjusted HR=1.87, CI=0.76–4.60, and 0.97, CI=0.66–1.41 for those hospitalized for CAP and non-infectious illnesses, interaction p=0.01).

Diabetes did not alter immune response after CAP hospitalization

In GenIMS, the circulating concentrations of inflammatory (TNF, IL-6, and IL-10), coagulation (antithrombin, factor IX, TAT complexes), and fibrinolysis (PAI-1 and D-dimer) biomarkers were similar among subjects with and without diabetes at ED presentation and over first week of hospitalization (Figure 2). No differences were seen in expression of CD120a, CD120b, HLA-DR, and TLR4 and TLR2 expression on monocytes between those with and without diabetes (Figure 3). Expression of TLR2 on granulocytes was higher among subjects with diabetes on day 1 and 3, but higher among those without diabetes on day 7 (p=0.04). At hospital discharge, those with diabetes had higher IL-6 concentrations compared to those without, but these differences were small (8.67 vs. 7.41 pg/ml, p=0.03) (Table 5). No differences were observed in other biomarkers at hospital discharge.

Plasma glucose concentrations

Plasma glucose was available at ED presentation in most GenIMS subjects (n= 1795, 94.7%). Those with diabetes had higher median plasma glucose compared to those without diabetes (190 mg/dl, interquartile range (IQR)=136–256 mg/dl vs. 117 mg/dl, IQR=99–143 mg/dl, p<0.0001).

Regardless of diabetes, non-survivors at 1 year had higher median blood glucose on ED presentation compared to survivors, but differences were small (130 mg/dl, IQR=103–177 mg/dl vs. 122 mg/dl, IQR=102–158 mg/dl, p=0.02). Hyperglycemia at ED presentation (glucose >200mg/dl) was associated with higher mortality within first year (HR=1.31, CI=1.01–1.69, p=0.03). However, results did not reach statistical significance when adjusted for age, sex, and race (HR=1.27, CI=0.98–1.64, p=0.06). Furthermore, the HRs were different when the analyses were stratified by presence or absence of diabetes (HR=0.89, CI=0.6–1.32, p=0.57 in subjects with diabetes and HR=1.4, CI= 0.98–2.11, p=0.06 in subjects without diabetes).

DISCUSSION

We found that diabetes was common and present in 20% of subjects with CAP. Compared to subjects without diabetes, those with diabetes had higher mortality within the first year after CAP and a fourth died at 1 year. The higher mortality was not confounded by higher burden of pre-existing cardiovascular disease and CKD. Furthermore, diabetes was associated with higher mortality following hospitalization for CAP compared to hospitalization for non-infectious illnesses. These results suggest that higher mortality within the first year among individuals with diabetes is attributable to the pneumonia hospitalization. Diabetes did not modify the immune response in a broad panel of circulating inflammatory, coagulation, fibrinolysis, and cell surface markers, suggesting that differences in immune response are unlikely to explain survival differences. Based on patterns of organ dysfunction observed during the hospital course and cause-specific mortality, the higher incidence of acute kidney injury and acceleration of underlying cardiovascular disease may mediate higher mortality within the first year after CAP among subjects with diabetes.

Our results showing survival differences due to diabetes after CAP hospitalization has several strengths. First, the higher hazards of death due to diabetes were similar in two cohorts, though results did not meet statistical significance in Health ABC due to the small number of CAP events. Second, we assessed survival differences after the first hospitalization event in Health ABC to avoid confounding due to preceding non-infectious illnesses, such as acute myocardial infarction, which are common and may be associated with worse survival in diabetes.[10] We also demonstrated an interaction between diabetes, reason for hospitalization, and mortality within the first year. Among those with diabetes, mortality was higher after CAP hospitalization compared to hospitalization for non-infectious illnesses. Third, survival differences persisted after adjusting for differences in chronic disease burden, such as cardiovascular disease and CKD. These results suggest that the higher mortality within the first year in diabetes can be attributed to the CAP hospitalization and cannot be explained by higher burden of pre-existing cardiovascular disease and CKD among those with diabetes.

We speculate that acceleration of pre-existing chronic disease may explain higher long-term mortality among those with diabetes. For instance, cardiovascular disease accounted for more than third of all deaths in individuals with diabetes. Pre-existing cardiovascular disease was more common among those with diabetes, which may be further accelerated by the acute infection. Early recognition or better management of atherosclerotic heart disease and concomitant risk factors, such as smoking and hyperlipidemia, may improve outcomes. We

showed that diabetes was associated with higher risk of acute kidney injury, which was associated with higher risk of 1-year mortality in our study and prior studies.[28] Acute kidney injury itself or its sequelae, chronic kidney disease, may lead to death by several mechanisms, including increased risk of cardiovascular disease and infections.

Our results may explain the conflicting results of prior studies which assessed the association between diabetes and survival after CAP. First, mortality was assessed at different time-points in prior studies. Our results suggest that, although the relative risk of death was similar at different time points, statistically significant differences are more likely to be seen in studies that assessed long-term outcomes due to fewer deaths at earlier time points.[3] Second, we examined the effect of diabetes on outcomes, conditional upon developing pneumonia. Prior population-based studies that showed higher incidence of death due to infections in subjects with diabetes could not assess whether these differences were due to increased susceptibility to infections or worse outcomes after infection.[5,6]

We showed a 1.3-fold higher risk of acute kidney injury among diabetic subjects, even among subjects without history of chronic kidney disease. Mechanisms underlying increased risk of AKI after CAP among diabetics are unclear. We speculate that the higher risk of acute kidney injury in diabetic subjects could be due to higher prevalence of subclinical kidney disease prior to pneumonia or due to higher risk of developing contrast-induced nephropathy.[29,30]

Although immune dysfunction in diabetes is well recognized, the lack of a clear influence of diabetes on a broad panel of biomarkers and cell-surface markers at multiple time points during hospitalization for CAP is remarkable. We observed differences in expression of TLR2 on granulocytes over time between subjects with and without diabetes, but the direction of this difference was not consistent over time and uncertain in terms of biologic significance. We chose inflammatory, coagulation, and fibrinolysis biomarkers because these biomarkers are altered in diabetes [11,31] and in human endotoxemia models by hyperinsulinemia and hyperglycemia.[12,14]. We chose TLR2 and TLR4 because expression of these cell-surface markers and their downstream mediators are upregulated in diabetes [16] and they play an important role in host response to infection and sepsis.[15] We performed serial measurements of these markers, including on presentation to the ED, when immune responses are least likely to be modified by therapeutic interventions. It is likely that diabetes may modify these markers prior to ED presentation or other mechanisms could be influenced by diabetes, such as alterations in neutrophil function, apoptosis, oxidative stress, and chemokines. However, the lack of effect of diabetes on the immune response in a broad panel of circulating biomarkers and cell-surface markers suggest that pre-existing diabetes does not influence the immediate host response to CAP, possibly because the responses elicited by pneumonia are much more profound than the relatively modest alterations produced by diabetes per se.

Our study has limitations. First, we assessed the effect of hyperglycemia, a potential mechanism of increased mortality, on ED presentation only, and serial glucose levels were not available. Second, we used different criteria to diagnose diabetes in both cohorts. Stringent criteria were used in Health ABC, including self-report and review of medication inventory, and fasting blood glucose in subjects who did not report diabetes. In GenIMS, we used a combination of self-report and review of medication inventory, and we may have misclassified some individuals as non-diabetic. The association between admission hyperglycemia and higher mortality only among those without diabetes in this cohort suggests that such a misclassification bias would skew the results towards the null and attenuate the hazards ratios between diabetes and long-term survival. Indeed, the HRs were slightly lower in GenIMS, suggesting that such a misclassification bias is unlikely to negate

our results. Finally, we could not assess whether our results were confounded due to differences in microbiologic etiology between those with and without diabetes. Although cultures were obtained in most subjects, we identified an etiologic agent in a small subgroup. The low yield of cultures in our study is consistent with previous large studies of CAP patients[32,33] and likely due to poor yield of current culture techniques. Larger studies will be necessary to understand differences in immune response for different etiologic agents.

In summary, once CAP occurs, those with diabetes were more likely to die over 1 year. The mechanism is unlikely to be due to alterations in immune response, at least as measured by a broad panel of circulating biomarkers and cell surface markers. The higher mortality may be due to worsening of pre-existing cardiovascular disease or higher risk of acute kidney injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

List of non-infectious causes of hospitalization in Health ABC study

Myocardial infarction

Angina/other ischemic disease

Congestive heart failure

Carotid artery disease

Peripheral arterial disease

Stroke or cerebrovascular accident (CVA)

Transient ischemic attack

Chronic obstructive pulmonary disease (COPD)/Emphysema/Asthma

Upper gastrointestinal bleeding

Lower gastrointestinal bleeding

Abdominal hernia

Benign prostatic hyperplasia (BPH)

Gallbladder disease

Cancer

Depression

Dementia

Osteoarthritis

Fracture

Neoplasms (benign)

Endocrine, nutrition, metabolic diseases

Diseases of blood and blood forming organs

Mental disorders (not dementia and depression)

Diseases of nervous system, other than stroke

Diseases of circulatory system, other than myocardial infarction, angina, and congestive heart failure

Diseases of respiratory system, other than COPD, asthma, emphysema

Diseases of digestive system, other than bleeding

Diseases of genitourinary system, other than BPH

Diseases of skin

Diseases of musculoskeletal and connective tissue, other than osteoarthritis

Ill defined symptoms and signs

Injury and poisoning, excluding fractures

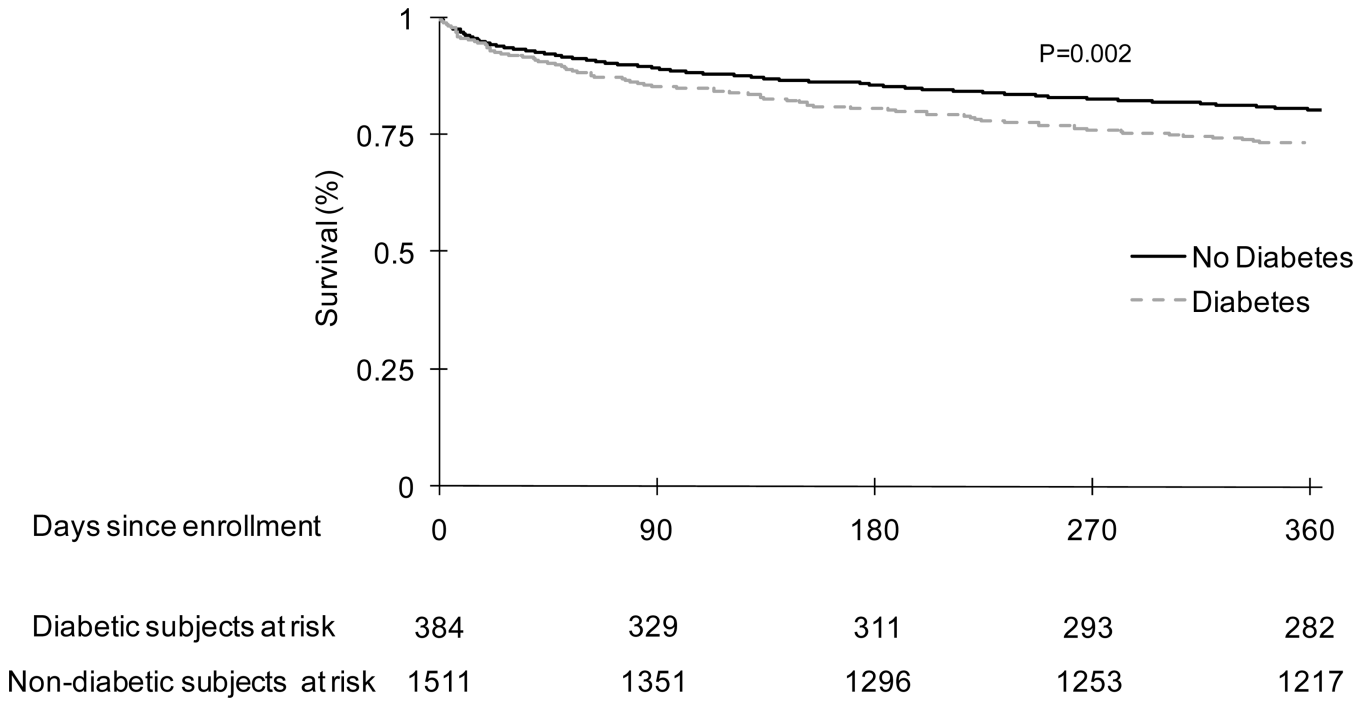
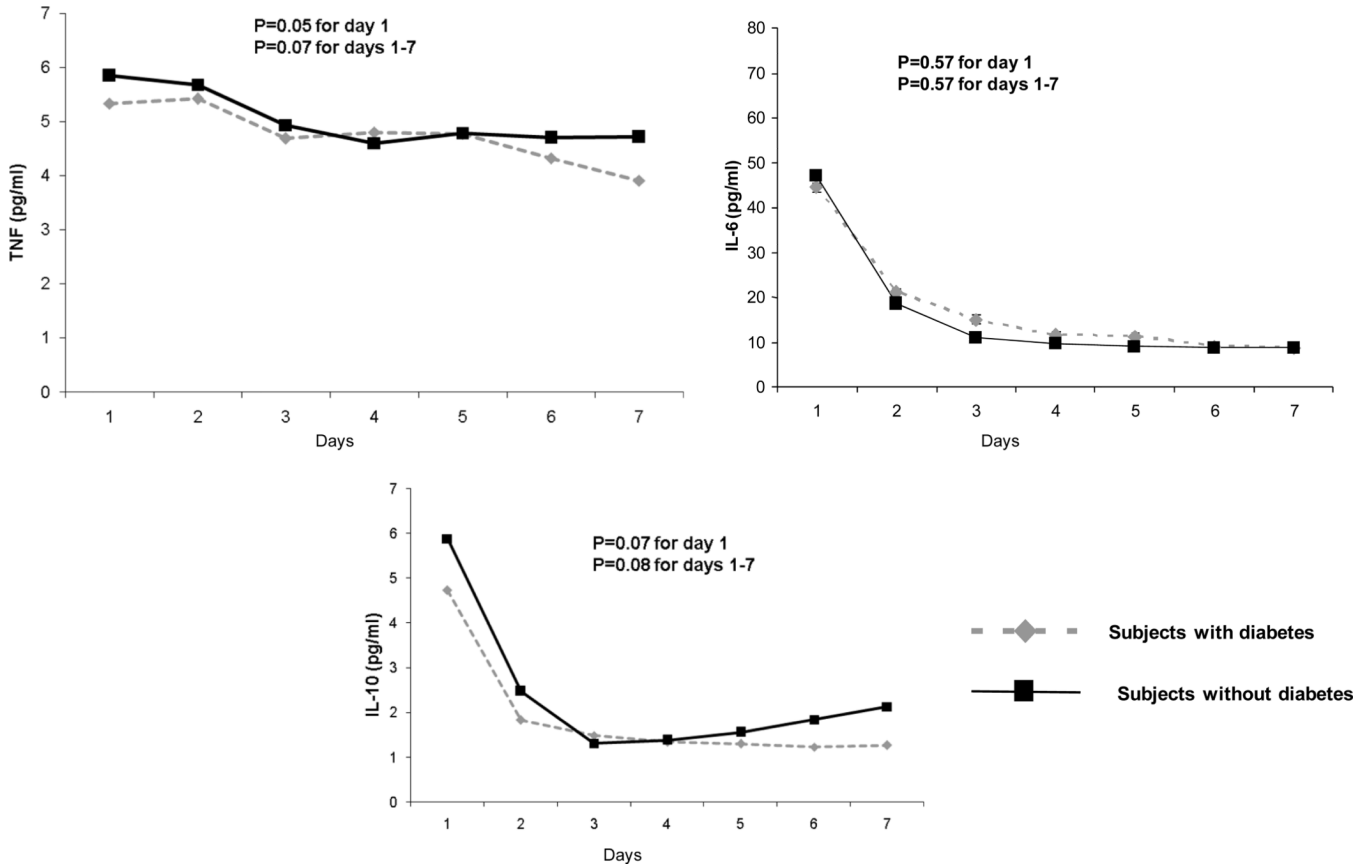


Figure 1.
Failure plots over 1 year showing higher risk of death for subjects with diabetes compared to those without diabetes following hospitalization for community acquired pneumonia in GenIMS.



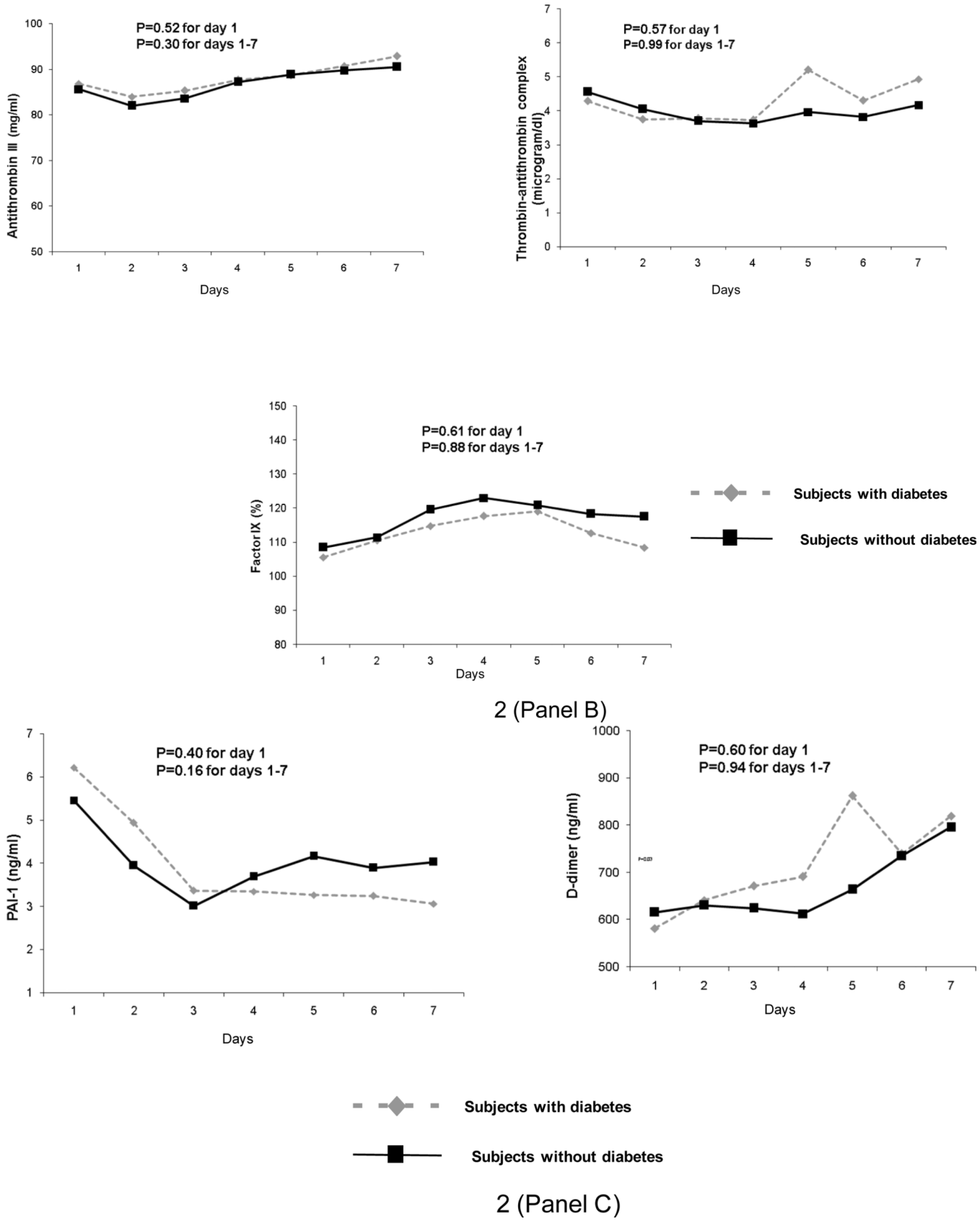


Figure 2.

No differences were observed in geometric means of inflammatory (tumor necrosis factor [TNF], interleukin (IL)-6, and IL-10 shown in Panel A), coagulation (Factor IX, antithrombin, and thrombin-antithrombin [TAT) complexes shown in Panel B), and fibrinolysis (plasminogen activator [PAI]-1 and D-dimer shown in Panel C) biomarkers in 1427, 734, and 734 subjects with and without diabetes, respectively. Biomarkers were measured on presentation to the emergency department and over the first week of hospitalization and p values are shown for these comparisons.

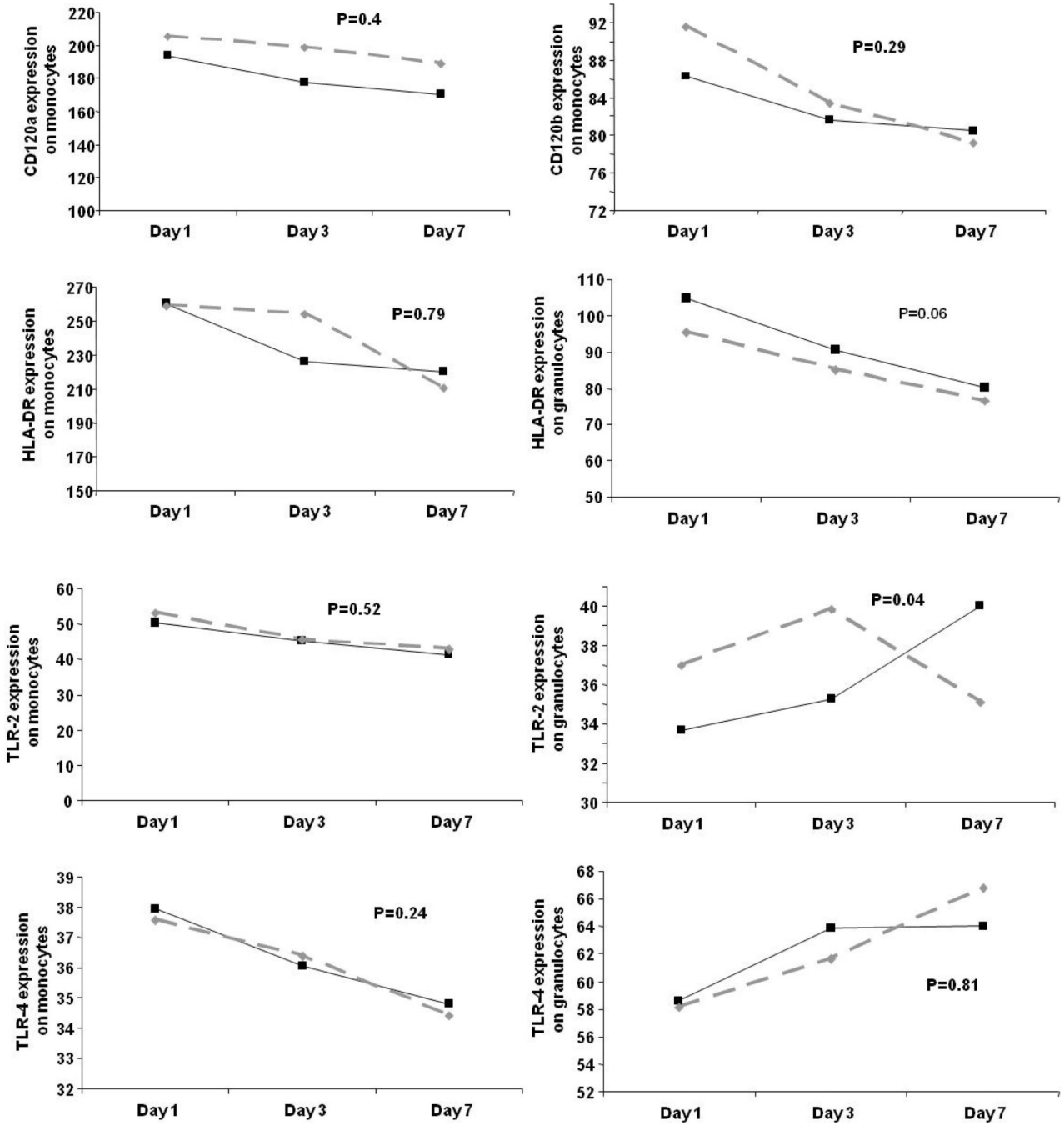


Figure 3.

No differences were observed in cell surface markers on presentation to the emergency department and on third and seventh day in subjects with and without diabetes in 624 subjects. Values for cell surface markers were reported as the mean channel fluorescence (MCF) of cells positive for a given cell surface marker. P values are shown for comparisons over time. Only expression of TLR2 on granulocytes appeared to be higher among subjects with diabetes on day 1 and 3 and were higher in those without diabetes on day 7 (p=0.04).

Table 1

Characteristics, eligibility criteria, and methods to determine clinical and outcome measures in Genetic and Inflammatory Markers of Sepsis (GenIMS) and Health, Aging, and Body Composition (Health ABC) cohorts.

Variable	GenIMS study	Health ABC study
Inclusion criteria	Age >18 years and clinical and radiologic diagnosis of community-acquired pneumonia	70–79 year community-dwelling older adults
Exclusion criteria	Transfer from another hospital, discharge from an acute care hospital within the previous ten days, diagnosis of pneumonia within the previous 30 days, chronic dependency on mechanical ventilation, cystic fibrosis, active pulmonary tuberculosis, admission for palliative care, prior enrollment in the study, incarceration, or pregnancy.	Actively treated cancer, severe cognitive impairment, or plans of leaving the area within three years.
Enrollment period	2001–2003	1997–1998
Ascertainment of outcome measures		
All-cause mortality	By study nurses during hospitalization and by National Death Index after hospital discharge	Semi-annual telephone follow-up, notification of death by proxy, spouse, relative, or friend, and review of obituaries in local newspaper. Once a death was reported, a copy of the death certificate was obtained in all cases to confirm death, and the next of kin or a pre-designated proxy was interviewed
Cause specific mortality	National Death Index codes	Not analyzed due to small sample size
Severe sepsis	Defined as pneumonia and organ dysfunction, based on the International Consensus Criteria[34]	Not assessed
Comorbid conditions		
Diabetes mellitus	Self-report and review of medications (use of oral hypoglycemic medications or insulin)	Self-report and review of medications (use of oral hypoglycemic medications or insulin) or fasting glucose ≥126 mg/dl[35]
Cardiovascular disease	Self-report and review of medications	Self-report and review of medications
Chronic kidney disease	Self report and pre-hospitalization creatinine measurements, if available. We also used creatinine measurements available during hospitalization to exclude chronic kidney disease	Creatinine measurements at enrollment and using the MDRD criteria[36]

Table 2

Demographic and clinical characteristics of all subject, stratified by diabetes, enrolled in Genetic and Inflammatory Markers of Sepsis (GenIMS) and Health, Aging, and Body Composition (Health ABC) study

Variable	GenIMS study (n=1895)			Health ABC study (n=1645)		
	All subjects	With diabetes (n=384)	Without diabetes (n=1511)	All subjects	With diabetes (n=299)	Without diabetes (n=1340)
Demographics						
Age, mean (sd, median)	67.2, (16.8, 72)	69.3, (14.5, 73)	66.7 (17.3, 71)	73.7 (2.8, 74)	73.7 (2.8, 73)	73.7 (2.8, 74)
Sex, female, n (%)	910 (48.0)	177 (46.1)	733 (48.5)	766 (46.7)	132 (44.1)	634 (47.3)
Race, white, n (%)	1,529 (80.7)	307 (80)	1,222 (80.1)	939 (57.2)	127 (42.4)	812 (60.6)
Health behaviors						
Ever smoked, n (%)	1,262 (66.6)	244 (63.5)	1,018 (67.4)	997 (60.9)	183 (61.6)	814 (60.8)
Chronic health conditions						
Respiratory disease, n (%)	718 (37.9)	148 (38.5)	570 (37.7)	320 (24.2)	64 (27.4)	256 (19.3)
Cardiovascular disease, n (%)	488 (25.7)	165 (42.9)	323 (21.3)*	438 (27.3)	97 (35.9)	341 (25.9)*
Chronic kidney disease, n (%)	92 (4.8)†	40 (10.4)	52 (3.4)*	403 (24.5)	102 (34.1)	301 (22.4)*
Cancer§, n (%)	94 (5.0)	22 (5.7)	72 (4.8)	-	-	-
HIV§, n (%)	37 (2.0)	1 (0.3)	36 (2.4)*	-	-	-

*p<0.05 for comparison among subjects with and without diabetes;

†Chronic kidney disease (CKD) diagnosis was available in 1571 (82.9%) subjects in GenIMS. In the remaining 324 subjects, CKD could not be ruled out based on history and because their plasma creatinine values were elevated on the first day of hospitalization for pneumonia,

§Health ABC excluded subjects with active cancer and none reported HIV.

Table 3

Risk of severe sepsis and organ dysfunction among subjects with and without diabetes*

Variable	With diabetes	Without diabetes	P value
Risk of severe sepsis, n (%)	133 (34.6)	449 (29.7)	0.06
Organ dysfunction, n (%)			
Respiratory	76 (19.8)	254 (16.8)	0.17
Cardiovascular	15 (3.9)	60 (4.0)	0.95
Renal	116 (30.2)	346 (22.9)	0.007
Liver	1 (0.3)	11 (0.7)	0.27
Neurologic	31 (8.1)	83 (5.5)	0.06
Coagulation	4 (1.0)	19 (1.3)	0.49

*Consensus criteria were used to define severe sepsis and acute organ dysfunction, except for renal dysfunction where the RIFLE criteria were used.[20,37]

Table 4

Unadjusted and adjusted hazard ratios (HR) with 95% confidence intervals (CI) for mortality over 1 year for subjects with diabetes compared to those without using Cox proportional hazards model

Variables	GenIMS study		Health ABC study				
	Pneumonia hospitalization		Pneumonia hospitalization		Hospitalization for non-infectious illness		Interaction p value*
	HR with 95% CI	p value	HR with 95% CI	p value	HR with 95% CI	p value	
Unadjusted	1.41 (1.12–1.76)	0.002	1.87 (0.76–4.6)	0.16	1.16 (0.8–1.6)	0.37	0.04
Adjusted for age, race, sex	1.32 (1.05–1.66)	0.01	1.82 (0.72–4.62)	0.2	1.08 (0.76–1.52)	0.65	0.04
Adjusted for age, race, sex, cardiovascular and kidney disease	1.3 (1.03–1.65)	0.02	1.8 (0.66–4.92)	0.25	1.06 (0.75–1.51)	0.7	0.09

* p value for interaction between diabetes and the reason for initial hospitalization (pneumonia and non-infectious illness) on mortality over 1 year

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Table 5

Association between inflammatory, coagulation, fibrinolysis markers (geometric means with standard deviation) at hospital discharge stratified by diabetes among 1808 subjects discharged alive from the hospital

Biomarker	With diabetes	Without diabetes	p-values
Inflammatory markers (pg/ml)*			
TNF	4.77 (2.12)	4.73 (2.65)	0.51
IL-6	8.67 (6.83)	7.41 (5.74)	0.03
IL-10	1 (18.45)	1.24 (11.92)	0.6
Coagulation markers*			
Factor IX activity (%)	111.02 (1.30)	119.14 (1.25)	0.1
Antithrombin (mg/ml)	91.77 (1.04)	91.50 (1.04)	0.87
TAT complexes (μg/ml)	4.25 (3.70)	3.58 (2.49)	0.25
Fibrinolysis markers (ng/ml)*			
D-dimer	623.79 (3.26)	600.86 (2.92)	0.67
PAI-1	3.54 (4.27)	3.72 (4.63)	0.62

*Inflammatory, coagulation, and fibrinolysis markers were available at hospital discharge in 1739, 892, and 892 subjects, respectively