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Polymorphisms in the *mannose binding lectin-2* gene and acute respiratory distress syndrome*

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Abstract

Objective—The variant alleles in the *mannose binding lectin-2 (MBL-2)* gene have been associated with MBL deficiency and increased susceptibility to sepsis. We postulate that the variant *MBL-2* genotypes are associated with increased susceptibility to and mortality in acute respiratory distress syndrome (ARDS).

Design—Nested case-control study.

Setting—Tertiary academic medical center.

Patients—Two hundred and twelve Caucasians with ARDS and 442 controls genotyped for the variant *X, D, B*, and *C* alleles of *codon -221*, *52*, *54*, and *57*, respectively.

Interventions—None.

Measurements and Main Results—Patients homozygous for the variant *codon 54B* allele (*54BB*) had worse severity of illness on admission ($p = .007$), greater likelihood of septic shock ($p = .04$), and increased odds of ARDS (adjusted odds ratio, 6.7; 95% confidence interval, 1.5-31) when compared with heterozygotes and homozygotes for the wild-type allele. This association with ARDS was especially strong among the 311 patients with septic shock (adjusted odds ratio, 12.0; 95% confidence interval, 1.9-74). Among the patients with ARDS, the *54BB* genotype was associated with more daily organ dysfunction ($p = .01$) and higher mortality (adjusted hazard rate, 4.0; 95% confidence interval, 1.6-10). Development of ARDS and outcomes in ARDS did not vary significantly with variant alleles of *codon -221*, *52*, and *57*, but the power to detect an effect was limited secondary to the low allele frequencies.

Conclusions—The *MBL-2 codon 54BB* genotype may be important in ARDS susceptibility and outcome. Additional studies are needed to confirm these findings in other populations.

Keywords

acute respiratory failure; genetic susceptibility; acute lung injury; molecular epidemiology

*See also p. 293.

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Although clinical predictors for the development to acute respiratory distress syndrome (ARDS) are well recognized, a minority of patients with these risks develop ARDS (1). Genetic susceptibility to acute lung injury may explain the observed interindividual differences in risk and in outcomes (2-4).

Mannose binding lectin (MBL) is a member of the collectin family important in the initiation of the lectin pathway of complement activation and opsonin-induced phagocytosis (5-7). The MBL protein is encoded by the *mannose binding lectin-2 (MBL-2)* gene on chromosome 10. It is now known that circulating MBL levels are due largely to three single nucleotide polymorphisms (SNPs) in *codon 52* (db SNP ID rs5030737), *54* (db SNP ID rs1800450), and *57* (db SNP ID rs1800451) in exon 1 and a promoter SNP at codon -221 (*MBLXY;* db SNP ID rs7096206) (7). The variant exon 1 alleles are known as *D, B,* and *C,* respectively, whereas the wild-type alleles are known collectively as the *A* allele. The variant alleles in exon 1 and the *X* allele in the *MBLXY* polymorphism have been found to be associated with serum MBL deficiency especially in individuals homozygous for the variant alleles (8,9).

In clinical studies, variant *MBL-2* alleles have been associated with increased susceptibility to meningococcemia (10), invasive pneumococcal infection (11), hepatitis B (12,13), severe acute respiratory syndrome (14), and other infections (8,9,15-17). In critical illnesses, two small studies have found an association between the variant *MBL-2* alleles and increased incidence of systemic inflammatory syndrome from both infectious and noninfectious causes, increased severity of sepsis, and/or increased mortality in sepsis (18,19). In both studies, the variant alleles were associated with serum MBL deficiency with the lowest levels found among those patients who were homozygous for the variant alleles.

Genes that are important in sepsis are likely to be relevant in ARDS because of the many common links between sepsis and ARDS. Sepsis is the leading cause of ARDS (1). Most patients who die from ARDS die of refractory infection and sepsis, not from respiratory failure (20-22).

We describe a nested case-control study of patients at risk for ARDS. We hypothesized that the *X* allele of the *MBLXY* polymorphism and the variant *D, B,* and *C* alleles of the *codon 52, 54,* and *57* in the *MBL-2* gene are associated with increased susceptibility to and increased mortality in ARDS.

MATERIALS AND METHODS

Study Population and Design

A schematic summary of the study design is illustrated in Figure 1. Details of the study have been described previously (23). Briefly, all admissions to the intensive care units (ICU) of the Massachusetts General Hospital (Boston, MA) were screened daily for study-defined clinical risk factor for ARDS as detailed in Table 1 (2,23). Exclusion criteria included age <18, diffuse alveolar hemorrhage, chronic lung diseases, directive to withhold intubation, immunosuppression except if secondary to corticosteroid, and treatment with granulocyte colony-stimulating factor. ICU admissions with one or more defined risks for ARDS and no exclusion criteria were eligible for the study. Enrolled patients were screened daily for the primary outcome of ARDS as defined by respiratory failure requiring intubation and fulfillment of American-European Consensus Conference criteria for ARDS as follows (23,24): a) presence of hypoxemia as evidenced by PaO_2/FIO_2 ≤200 mm Hg; b) presence of bilateral infiltrates on chest radiographs; and c) absence of left atrial hypertension as evidenced by pulmonary arterial occlusion pressure ≤18 mm Hg or lack of notation for congestive heart failure as a problem in the progress note.

Nested within the prospective cohort, a case-control study was designed (Fig. 1). All patients who did not develop ARDS during their hospitalization with no prior history of ARDS or prior enrollment into the study were selected as controls. The Human Subjects Committees approved the study, and informed written consent was obtained from all subjects or their appropriate surrogates.

Baseline clinical information was collected for all subjects on admission to ICU. Vital signs and laboratory variables in the first 24 hrs after ICU admission were collected for calculation of Acute Physiology and Chronic Health Evaluation (APACHE) III. ARDS patients were followed for the secondary outcomes of all-cause 60-day mortality and daily multiple organ dysfunction score (MODS) for 28 days as defined according to Brussels Organ Dysfunction Score (23,25). Respiratory failure was not included in the calculation of the organ dysfunction score in the ARDS patients.

Recollection and re-entry of the clinical data from 89 (13%) subjects selected at random revealed a data-entry error rate of 1% and a data collection error rate of 2.8%. Missing physiology data occurred in <13% of patients. Similar to the APACHE III study, missing physiology values were assumed to be within the normal range. All other missing data were coded as missing. All data were collected onto clinical data forms and entered into an ACCESS data-base at the Harvard School of Public Health.

Genotyping Methods

Blood (10 mL) was collected for DNA extraction and polymerase chain reaction amplification. Genotyping was done on the Sequenom MassARRAY for *MBLXY* and *codon 57* and by polymerase chain reaction/restriction fragment length polymorphism for *codon 54* (9) and Taqman for *codon 52*. The genotyping for a random 5% of samples was repeated using alternative methods for quality control. All genotyping results were interpreted by two separate research staff. Research personnel were blinded to the case-control status or genotype of the subjects.

Haplotypes were generated from the genotype results using the Partition Ligation-Expectation Maximization (PL-EM) version 1.0 (26), as in other association studies (27) that reconstruct individual probabilities for individual phasing accuracy based on unphased genotype data. Only those 589 patients with complete genotyping data for the four *MBL2* polymorphisms were used to infer haplotypes.

Statistical Analysis

Conformity to Hardy-Weinberg equilibrium and Lewontin's D' for linkage disequilibrium was determined using SAS/Genetics (version 9.0) (28). Univariate analysis was performed using Fisher's exact test, analysis of variance, or Wilcoxon rank-sum tests. Kaplan-Meier curves for 60-day ARDS survival were compared using the log-rank test. ARDS patients who were lost to follow-up, who were discharged home alive before 60 day, or who survived to 60 days were censored at discharge or at 60 days if known to be alive.

Multivariate analyses included logistic regression for development of ARDS and Cox proportional hazard models for ARDS survival. Backward selection algorithms were used to determine possible confounders. The final multivariate model include the gene effect, results from backward elimination, and clinically relevant variables such as APACHE III score. A Hosmer-Lemeshow test was used to evaluate logistic regression model fit (29). Interactions with the *MBL-2* genotypes were tested with interaction terms, and no significant interactions were found. Only APACHE III scores deviated from proportional hazard assumption as indicated by time varying covariates ($p = .04$), so the final model for ARDS survival was stratified by APACHE III score by quartiles.

Daily MODS after development of ARDS was compared using linear mixed effects models assuming an unstructured covariance matrix using the Proc Mixed procedure in SAS. The fixed factors included the polymorphisms and potential confounders such as age, trauma, baseline APACHE III scores, treatment with corticosteroids before admission, liver failure, transfusion, and septic shock. The number of days after development of ARDS was considered to be a random factor. To evaluate whether the MODS score varied significantly by genotype during the course of ARDS, an interaction term between genotype and time was included in the model. For patients discharged from the hospital within 28 days of ARDS, their last available MODS score before discharge was assigned to all subsequent days. For ARDS patients who died within 28 days of ARDS, the maximal score was assigned to all days subsequent to death. Ventilator-free days in ARDS were calculated as previously described (30). All analyses were conducted using SAS version 9. A p value of .05 was considered statistically significant.

Assuming an α-error of .05, 80% power, and genotype frequencies of 24% for the *MBLXY X* allele and 12%, 26%, and 3% for variant *52D*, *54B*, and *57C*, respectively (9,31), a study with 212 cases and 442 controls would have a minimum detectable odds ratios of 1.6 for *MBLXY* and 1.9, 1.7, and 2.9 for *codon 52*, *54*, and *57*, respectively.

RESULTS

Patient Population

Between September 9, 1999, and October 15, 2002, 752 patients were enrolled including 237 ARDS patients and 477 controls selected and genotyped for the *MBL2* polymorphisms in the case-control study (Fig. 1). All subsequent analyses were restricted to the 212 Caucasian cases and 442 Caucasian controls.

Clinical risk factors for ARDS and baseline characteristics on admission to the ICU are shown in Tables 2 and 3. Variables in the final model for development of ARDS included direct pulmonary injury ($p < .001$), trauma ($p < .001$), age ($p = .002$), female gender ($p = .04$), diabetes ($p = .003$), platelets ≤80,000/mm ($p = .003$), blood transfusion ($p < .001$), septic shock ($p = .1$), and APACHE III score ($p = .6$). The Hosmer-Lemeshow goodness-of-fit statistic for the final model was 9.3 with 8 degrees of freedom ($p = .3$), indicating no significant lack of fit of the model.

Genotype Analyses and Development of ARDS

The variant allele frequency in the study was 8% for *codon 52*, 15% for *codon 54*, 1.5% for *codon 57*, and 17% for *MBLXY*, which is similar to prior studies (8,10,11,32). Genotype failure for *codon 52*, *54*, *57*, and *MBLXY* occurred in 38 (6%), 0 (0%), 15 (2%), and 13 (2%) patients, respectively. Genotype frequencies among cases and controls are shown in Table 4. Among the 212 patients with ARDS, the observed frequency for the *codon 54BB* genotype was higher than that predicted by Hardy-Weinberg equilibrium. However, this was not statistically significant ($p = .4$), given the smaller number of patients with ARDS. Among the 442 controls, the genotype frequency for *codon 54* deviated from that predicted by Hardy-Weinberg equilibrium ($p = .03$). This is unlikely to be due to genotyping error as there was no discrepancy on repeat genotyping. However, controls in this study were not healthy, and patients homozygous for the *codon 54B* genotype were more likely to have septic shock on ICU admission as a predisposing injury for ARDS (eight of ten [80%] vs. 303 of 643 [47%]; $p = .04$). An association between the *codon 54B* genotype and the underlying condition among the controls may explain the deviation from Hardy Weinberg equilibrium.

Among cases and controls, patients homozygous for the *MBL-2 codon 54* variant *B* allele had significantly greater severity of illness, as indicated by APACHE III score, than patients homozygous or heterozygous for the wild-type *A* allele ($p = .007$) (Fig. 2). Development of ARDS varied with the *codon* 54 genotype ($p < .05$), with patients who were homozygous for the variant *codon 54B* allele having an increased odds of developing ARDS compared with homozygotes or heterozygotes for the wild-type *A* allele (odds ratio [OR], 5.0, 95% confidence interval [CI], 1.3-20; $p = .02$) (Fig. 3). On multivariate analyses after adjustment for age, gender, APACHE III, septic shock, trauma, direct pulmonary injury, hematologic and hepatic failure, and transfusion, the *codon 54B* genotype was still significantly associated with development of ARDS (OR_{adj}, 6.7; 95% CI, 1.5-31; $p = .01$). When the analysis was restricted to the 311 patients admitted to the ICU with septic shock, the association between the *54BB* genotype and ARDS was even stronger (OR_{adj}, 12.0; 95% CI, 1.9 -74; $p = .008$).

Because of the linkage between the *MBLXY Y* allele and the *codon 54* variant *B* allele, patients homozygous for the *MBL Y* allele had higher APACHE III scores on admission to the ICU compared with patients who were homozygous or heterozygous for the *X* allele (mean 83 [SD 25] vs. 73 [25]; $p = .04$). However, the frequency of the variant alleles of *MBL XY, codon 52*, or *codon* 57 on the *MBL-2* gene did not vary significantly with ARDS (Table 4). When the variant alleles of *codon 52, 54*, and *57* were examined together as the variant *O* allele, carriage of the *O* allele was not associated with ARDS (OR_{adj}, 0.96; 95% CI, 0.66-1.4)

The four polymorphisms are in complete linkage disequilibrium (D' = 1.0). Consistent with other reports (14,18,33), the variant *codon 54 B* allele occurred only with the *Y* allele of the *MBLXY* polymorphism. Five haplotypes were identified with probability >99%. Haplotype analyses are similar to the preceding results. Only the *ABAY* haplotype was associated with the development of ARDS (Table 5).

Outcomes in ARDS

The 60-day mortality was 46% (98 of 211) for ARDS patients. Clinical risks for ARDS and baseline characteristics between survivors and non-survivors of ARDS are shown in Tables 2 and 3. Consistent with prior reports, survivors and nonsurvivors did not differ in Pao_2/FIo_2 ($p = .4$), compliance ($p = .4$), or Lung Injury Score ($p = .5$). However, non-survivors had significantly fewer ventilator-free days than survivors (median 0 days [25-75% 0-0] vs. 7 days [25-75% 2-14]; $p < .001$). The 60-day mortality in ARDS varied significantly with the *codon 54* genotype ($p = .03$) (Table 4) and the *ABAY* haplotype (Table 5). ARDS patients homozygous for the variant *B* allele had decreased survival compared with ARDS patients who were homozygous or heterozygous for the wild-type allele (hazard ratio [HR], 3.1; 95% CI, 1.4 -7.2; $p = .007$) (Fig. 4). Among controls, the *codon* 54BB genotype was not significantly associated with ICU mortality (HR_{adj}, 7.4; 95% CI, 0.90-61.3), although the power to determine an association was limited given that there were only three patients with the *BB* genotype who did develop ARDS. ARDS patients homozygous for the variant *codon 54B* allele had a nonsignificant trend to greater severity of illness on admission ($p = .08$) and significantly greater daily multiple organ dysfunction score after development of ARDS even after adjustment for potentially important variables (Fig. 5). On multivariate analysis, the *codon 54BB* genotype remained associated with increased mortality compared with ARDS patients who were homozygous for the wild-type *A* allele (HR_{adj}, 4.0; 95% CI, 1.6-10; $p = .003$).

The 60-day mortality in ARDS did not vary significantly with the *MBL-2* polymorphisms at *MBLXY, codon 52*, or *codon 57* (Table 4) or when examined together as the variant *O* allele (HR_{adj}, 1.0; 95% CI, 0.68-1.6).

DISCUSSION

In a study of prospectively enrolled ICU patients with clearly defined ARDS risk factors, we found significant associations between the *BB* genotype of the *MBL-2 codon 54* polymorphism and increased severity of illness on admission, increased development of ARDS, more multiple organ failures after development of ARDS, and increased mortality in ARDS. This study has a number of strengths. The prospective determination of ARDS using the American-European Consensus Conference definition helps minimize phenotype misclassification. In addition, clearly defined at-risk controls were used in this study. Using critically ill controls who have the opportunity to develop the outcome is more clinically relevant than using healthy individuals. Although this may bias the study toward the null, using at-risk controls also reduces the confounding from any possible association between the gene and the risk condition such as sepsis or pneumonia.

Several other studies have found the variant *O* allele (*codon 52D*, *codon 54B*, or *codon 57C* allele) to be associated with infections, systemic inflammatory responses, or other disease states in a codominant (AO and OO vs. AA genotype) (8,15,18,34,35) or recessive model (OO vs. AO and AA genotype) (11,10,9,36). We did not find any associations between ARDS and the *MBLXY X* allele or the variant alleles for *codon 52* and *57*. However, it is important to note that, given the low variant allele frequency, our power to detect an association for the *codon 52* and *57* was limited. Nevertheless, *codon 54* may be very significant in the *MBL-2* gene. The variant *codon 54B* allele is more prevalent than *codon 52* and *codon 57* in the Caucasian populations. Individuals with the *codon 54B* allele demonstrate even lower MBL protein concentration than individuals with the *52D* allele (33), and what MBL protein is produced was found to be incapable of activating the classic complement pathway (37). Of the four polymorphisms, *codon 54B* has been independently found to be associated with increased susceptibility to infection (12,14,16,17,38).

It is not clear why patients with the *codon 54 BB* genotype may be at increased risk of developing and dying of ARDS in this study. Homozygotes for the variant *MBL-2* alleles have been associated with a low circulating MBL (9,18). It is possible that patients with low MBL may be more susceptible to multilobar pneumonia or more severe septic shock.

Alternatively, it is possible that MBL may influence the inflammatory response to the initial injury in critical illnesses (7). MBL has been shown to decrease tumor necrosis factor release (39) and stimulate the production of anti-inflammatory cytokines such as interleukin-10 (40), but MBL modulation of TNF may be dose dependent. At low doses of MBL, increasing MBL concentration increases production of proinflammatory cytokines. But at higher concentrations, increasing MBL suppresses these inflammatory cytokines (41). It is possible that in critically ill patients with the low or defective MBL-producing genotypes, circulating MBL may not be sufficiently high or effective enough to switch from enhancement to suppression of the proinflammatory cytokine response. Unfortunately, the functional significance of the *MBL-2* polymorphisms and the inflammatory response of these patients in this study could not be examined. Additional studies are clearly needed to investigate the possible mechanisms by which *MBL-2* genotypes may be important in acute lung injury.

Among the controls in this study, the genotype frequency of the *codon 54* polymorphism deviated from that predicted by Hardy-Weinberg equilibrium. This is unlikely to be due to genotyping error. The genotype frequencies found here compare well with those reported in other populations. In addition, repeat genotyping in a random subset of patients revealed no discrepancy in genotype results. It is more likely that deviation from Hardy-Weinberg occurred because of a possible association between the *codon 54* polymorphism and the

underlying condition requiring ICU admission such as sepsis. Indeed, patients in this study who were homozygous for the variant *codon 54B* allele were more likely to have septic shock on admission to the ICU. The association found between the *codon 54 MBL-2* polymorphism and ARDS cannot be attributed to this association between *54B* and septic shock, a risk factor for ARDS. The association between the *54BB* genotype and ARDS was actually strengthened when the analyses were restricted to those patients with septic shock.

Given the low allele frequency for *codon 54*, only ten patients were homozygous for the variant *B* allele in the study and no adjustment for multiple comparisons were made. However, the findings in this report are unlikely to be due to type I error. The polymorphism was chosen *a priori* as a candidate in ARDS based on previous studies supporting its role in infection and critical illnesses. The results are consistent with our hypothesis and with previous reports on infection and sepsis. Additionally, the findings are supported by results of secondary out-comes such as septic shock, severity of illness, and organ failure after development of ARDS. Nevertheless, as is true of all genetic association studies, our findings will need to be confirmed in other populations.

We recognize some other limitations to our study. The functional significance of the *MBL-2* polymorphisms was not evaluated in this study. Because of the study design, the results may not be generalizable to the community setting, to immunocompromised hosts or patients without risk factors for ARDS, or with different clinical risks for ARDS. In addition, the analyses were restricted to Caucasians, which reduces the possibility of confounding from ethnicity (42) but does not permit extrapolation of the results to other ethnic groups.

CONCLUSIONS

We report an association between the *MBL-2 codon 54* genotype and severity of illness, septic shock, and development of ARDS. The *MBL-2 codon 54* genotype may also be associated with multiple organ dysfunction and mortality in ARDS. Additional studies are needed to confirm these findings in other populations with other risk factors.

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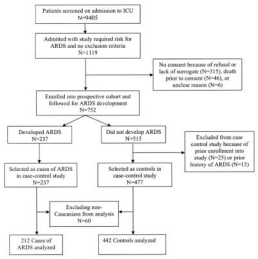

Figure 1.
Flow diagram of study design and patient selection for case-control study. *ICU*, intensive care unit; *ARDS*, acute respiratory distress syndrome.

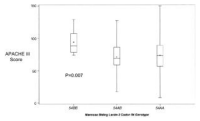

Figure 2.
Acute Physiology and Chronic Health Evaluation (*APACHE*) III score on admission to the intensive care unit by genotype for the *mannose binding lectin-2 codon 54* polymorphism among all 654 cases and controls. The box denotes the interquartile range (25-75%), the horizontal line in the box indicates the median, + designates the mean, and error bars indicate the 95% confidence intervals.

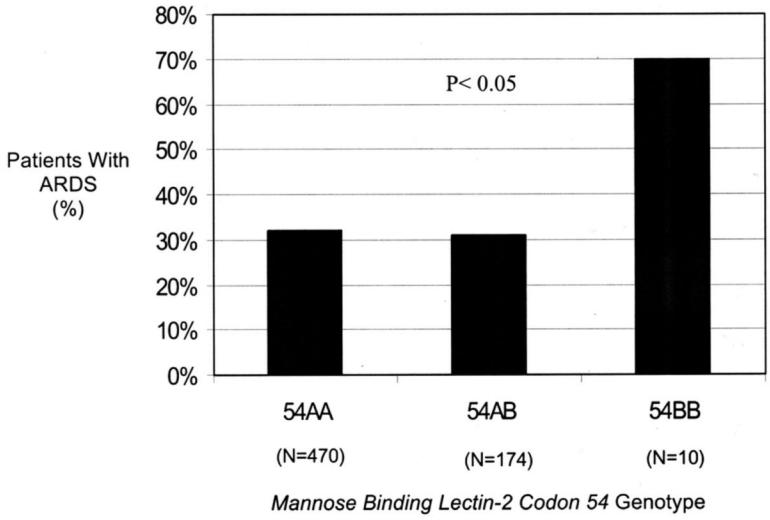

Figure 3.
The *mannose binding lectin-2 codon 54* genotype and the percentage of patients with acute respiratory distress syndrome (*ARDS*) with each genotype. The *p* value was calculated from Fisher's exact test.

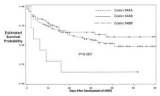

Figure 4.
Estimated survival probability in the 212 patients with acute respiratory distress syndrome (*ARDS*) by the *mannose binding lectin-2 codon 54* genotype.

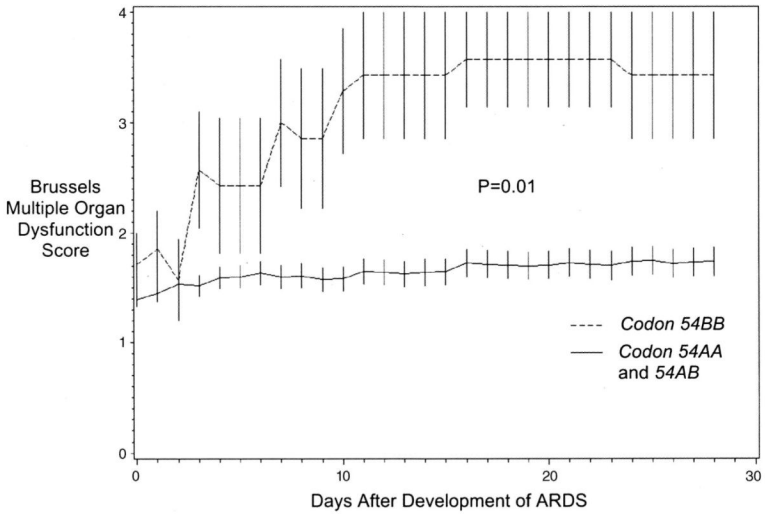

Figure 5.

Daily Brussels multiple organ dysfunction score for each day after development of acute respiratory distress syndrome (*ARDS*) for the 212 patients with ARDS after adjustment for potentially important variables such as age, trauma as a risk factor for ARDS, Acute Physiology and Chronic Health Evaluation III scores, history of treatment with corticosteroids, liver failure, transfusion, and septic shock.

Table 1

Study required risk factors for acute respiratory distress on admission to intensive care unit

Sepsis: As defined by the Society of Critical Care Medicine (42) to be a known or suspected source of systemic infection and at least two of the following: a) temperature >38°C or <36°C; b) heart rate >90 beats/min; c) respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg; d) WBC count >12,000/mm^3, <4000/mm^3, or >10% bands
Septic shock: Fulfill requirements for sepsis and one of the following: a) SBP <90 mm Hg or reduction of ≥40 mm Hg from baseline for ≥30 mins unresponsive to 500 mL of fluid resuscitation; b) need for vasopressors to maintain SBP ≥90 mm Hg or within 40 mm Hg of baseline
Pneumonia: Fulfill two or more of the following: a) new infiltrate on chest radiograph; b) temperature >38.3°C or <36.0°C or WBC >12,000 or <4000 or >10% bandemia; c) positive microbiologic culture
Trauma: Defined as multiple fractures and/or pulmonary contusions. Multiple fractures are defined as a fracture of two long bones, an unstable pelvic fracture, or one long bone and a pelvic fracture. Pulmonary contusion is defined as infiltrates on chest radiographs within 8 hrs of admission to the emergency room and evidence of blunt trauma to the chest such as fractured ribs or ecchymosis overlying the infiltrate.
Multiple transfusions: Defined as receiving ≤8 units of packed red blood cells within 24 hrs
Aspiration: Defined as witnessed or documented aspiration event or the retrieval of gastric contents from the oropharynx, endotracheal tube, or bronchial tree

WBC, white blood cell; SBP, systolic blood pressure.

Table 2

Clinical risk factors for acute respiratory distress syndrome (ARDS) between cases and controls and survivors and nonsurvivors in ARDS

	Development of ARDS No. (%)			Mortality in ARDS No. (%)		
Risk for ARDS[a]	**Controls (n = 442)**	**Cases (n = 212)**	***p* Value**	**Survivors (n = 114)**	**Nonsurvivors n = 98)**	***p* Value**
Sepsis syndrome	168 (38)	67 (32)	.1	44 (39)	23 (23)	.03
Pneumonia source	87 (20)	52 (25)	<.001	33 (29)	19 (19)	.6
Extrapulmonary source	81 (18)	15 (7)		11 (10)	4 (4)	
Septic shock	196 (44)	115 (54)	.02	52 (46)	63 (64)	.009
Pneumonia source	89 (20)	79 (37)	<.001	36 (32)	43 (44)	>.9
Extrapulmonary source	107 (24)	36 (17)		16 (14)	20 (20)	
Trauma	40 (9)	9 (4)	.04	8 (7)	1 (1)	.04
Multiple transfusions	51 (12)	26 (12)	.8	13 (11)	13 (13)	.7
Aspiration	34 (8)	24 (11)	.1	13 (11)	11 (11)	>.9
>1 Risk for ARDS	48 (11)	29 (14)	.3	16 (14)	13 (13)	>.9
Direct pulmonary injury[b]	211 (48)	144 (68)	<.001	79 (69)	65 (66)	.7
Indirect pulmonary injury[c]	231 (52)	68 (32)		35 (31)	33 (34)	

[a] Numbers of controls and cases with each risk add up to >654 patients because of multiple risks in 75 patients

[b] pneumonia, aspiration, or pulmonary contusions were categorized as direct pulmonary injury

[c] sepsis from an extrapulmonary source, trauma without pulmonary contusions, and multiple transfusions were categorized as indirect pulmonary injury. Patients (n = 69) with both direct and indirect pulmonary injuries were considered to have direct pulmonary injury.

Table 3

Baseline characteristics between patients with acute respiratory distress syndrome (ARDS) and controls and survivors and nonsurvivors in ARDS

	Development of ARDS			Mortality in ARDS		
	Controls (n = 442)	**Cases (n = 212)**	***p* Value**	**Survivors (n = 114)**	**Nonsurvivors (n = 98)**	***p* Value**
Females, n (%)	179 (41)	101 (48)	.09	50 (44)	51 (52)	.3
Age, median (range)	69 (18-94)	65 (18-97)	.05	57 (18-89)	73 (22-97)	<.001
APACHE III median (range)[a]	64 (14-130)	68 (8-136)	.08	69 (8-115)	88 (29-150)	<.001
Diabetes, n (%)[b]	116 (26)	34 (16)	.004	20 (18)	14 (14)	.6
History of alcohol abuse, n (%)	42 (10)	27 (13)	.2	10 (9)	17 (17)	.07
Tobacco abuse, n (%)[c]	217 (49)	105 (50)	.4	57 (50)	48 (49)	.7
Chronic liver disease, n (%)[b]	18 (4)	12 (6)	.4	4 (4)	8 (8)	.2
End-stage renal disease, n (%)	23 (5)	6 (3)	.2	2 (2)	4 (4)	.4
History of steroid use, n (%)	37 (8)	20 (9)	.7	6 (5)	14 (14)	.03
Transfusion of PRBC, n (%)	218 (49)	133 (63)	.001	64 (56)	69 (70)	.03
Number of PRBC transfused, median (range)	0 (0-74)	2 (0-63)	.005	1 (0-31)	2 (0-63)	.01
Systolic BP <90 mm Hg, n (%)	305 (69)	163 (77)	.04	85 (75)	78 (80)	.4
Creatinine <2.0 mg/L, n (%)	150 (34)	65 (31)	0.4	28 (25)	37 (38)	.05
Bilirubin <2.0 mg/dL, n (%)	53 (12)	39 (18)	.03	14 (12)	25 (26)	.02
Hematologic failure (platelets ≤80,000/mm), n (%)	60 (14)	47 (22)	.007	20 (18)	27 (28)	.1

APACHE, Acute Physiology and Chronic Health Evaluation; PRBC, packed red blood cells; BP, blood pressure.

[a] For development of ARDS, APACHE III scores for patients and controls were calculated without the PaO_2/FIO_2 component; for survivors and nonsurvivors in ARDS, APACHE III scores were calculated with all components

[b] chronic health information was missing on one patient and two controls

[c] tobacco history was missing in 56 (26%) patients and 97 (22%) controls.

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Table 4

Genotype frequencies for the *mannose binding lectin-2* (*MBL2*) *codon 52, 54, 57,* and *MBLXY* polymorphisms among patients and controls and among survivors and nonsurvivors in acute respiratory distress syndrome (ARDS)

	Percentage of Patients (No.)					
Polymorphism	**Controls (n = 442)**	**Cases (n = 212)**	***p* Value**	**Survivors in ARDS (n = 144)**	**Nonsurvivors in ARDS (n = 98)**	***p* Value**
Codon 52						
52AA	82 (345)	86 (170)		90 (97)	82 (73)	
52AD	17 (72)	14 (27)	.4	10 (11)	18 (16)	.1
52DD	0.5 (2)	0 (0)		0	0	
Codon 54						
54AA	72 (319)	71 (151)		68 (78)	74 (73)	
54AB	27 (120)	25 (54)	<.05	31 (35)	19 (19)	.03
54BB	0.7 (3)	3.3 (7)		1 (1)	6 (6)	
Codon 57						
57AA	97 (418)	98 (205)		98 (111)	97 (94)	
57AC	3 (12)	2 (5)	>.9	2 (2)	3 (3)	.5
57CC	0.2 (1)	0 (0)		0	0	
MBLXY						
YY	71 (306)	65 (136)		67 (75)	64 (61)	
XY	26 (111)	29 (60)	.2	26 (29)	32 (31)	.5
XX	3 (15)	6 (12)		7 (8)	4 (4)	

Fisher's exact test *p* values are reported.

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Table 5

Haplotype frequencies for the *mannose binding lectin-2* (*MBL-2*) *codon 52*, *54*, and *57* and *MBLXY* polymorphisms and development of acute respiratory distress syndrome (ARDS) and 60-day mortality in ARDS

		Development of ARDS				Mortality in ARDS			
		Patients With ARDS with Genotype/Total No. of Patients with Genotype (%)				Nonsurvivors with Genotype/All ARDS Patients with Genotype (%)			
		No. of Copies of Haplotype				No. of Copies of Haplotype			
Haplotype[a]	**Haplotype Frequency**[b]	**0**	**1**	**2**	***p* Value**	**0**	**1**	**2**	***p* Value**
AAAY	0.59	42/107 (39)	88/285 (30)	62/197 (31)	.2	20/42 (48)	41/86 (48)	24/62 (39)	.5
AAAX	0.16	124/406 (31)	55/158 (35)	11/25 (44)	.3	54/124 (44)	27/55 (49)	4/11 (36)	.7
DAAY	0.09	165/496 (33)	25/91 (27)	0/2 (0)	.4	70/165 (42)	15/25 (60)	0	.1
ABAY	0.14	134/422 (32)	49/157 (31)	7/10 (70)	<.05	63/134 (47)	16/49 (33)	6/7 (86)	.02
AACY	0.02	185/571 (32)	5/17 (29)	0/1 (0)	>.9	82/185 (44)	3/5 (60)	0	.7

[a] Haplotypes consist of *codon 52*, *codon 54*, *codon 57*, and *MBLXY*. The variant alleles for *codon 52*, *54*, and *57* are *D*, *B*, and *C*, respectively. The wild-type allele is known collectively as the *A* allele

[b] haplotype frequency among controls only. The *p* values from Fisher's exact test for each haplotype are presented. Only the *ABAY* haplotype was associated with development of ARDS ($p < .05$) and mortality in ARDS ($p = .02$).