Published in final edited form as:
Eur Respir J. 2006 April ; 27(4): 674–681. doi:10.1183/09031936.06.00046405.

Interleukin-10 polymorphism in position -1082 and acute respiratory distress syndrome

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Abstract

The GG genotype of the interleukin (IL)-10 promoter polymorphism in position -1082 (*-1082GG*) has been associated with increased IL-10 production. The current authors hypothesised that the *-1082GG* genotype is associated with the development of, and outcomes in, acute respiratory distress syndrome (ARDS).

A nested case-control study was conducted in 211 Caucasian cases of ARDS and 429 controls who were admitted to an intensive care unit with sepsis, trauma, aspiration or massive transfusions. Cases were followed for organ failure and 60-day mortality.

The *-1082GG* genotype was associated with the development of ARDS, but only in the presence of a significant interaction between the *-1082GG* genotype and age. Among patients with ARDS, the *-1082GG* genotype was associated with decreased severity of illness on admission, lower daily organ dysfunction scores and lower 60-day mortality.

In conclusion, the high interleukin-10-producing *-1082GG* genotype may be associated with variable odds for acute respiratory distress syndrome development depending on age. Among those with acute respiratory distress syndrome, the *-1082GG* genotype is associated with lower mortality and organ failure. Further studies are needed to confirm these findings.

Keywords

Acute respiratory distress syndrome; interleukin-10; mortality; polymorphism

Current understanding of why some individuals develop and die from acute respiratory distress syndrome (ARDS) while others do not is incomplete. Although clinical risks such as sepsis and trauma are well recognised, only a minority of patients with these risks develop ARDS [1]. Genetic polymorphisms important in innate immunity, pulmonary defence and inflammatory response have been found to be associated with the development of and outcomes in ARDS, sepsis and pneumonia [2-5], suggesting that genetic variation may

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explain some of the observed inter-individual differences in risk and outcomes in critical illnesses, such as ARDS.

Interleukin (IL)-10 is an important anti-inflammatory cytokine that modulates pro-inflammatory cytokines, such as tumour necrosis factor (TNF)-α, as well as synthesis of nitric oxide, apoptosis of inflammatory cells and suppression of macrophage activation [6]. IL-10 attenuates the pro-inflammatory response in sepsis and reduces mortality in some animal models [7,8], but not in others [9]. In humans, elevated circulating IL-10 has been associated with septic shock [10], severity of injury [11,12] and mortality [13,14]. Among patients with ARDS, the studies have been mixed. Lower levels of IL-10 were found in patients with ARDS compared with critically ill non-ARDS patients [15]. Among ARDS patients, high plasma IL-10 but low bronchoalveolar lavage concentration of IL-10 correlated with increased mortality [16,17].

It has been reported that 50-75% of the variation in IL-10 production is genetically controlled [18,19]. In the *IL-10* gene, a G to A single nucleotide promoter polymorphism at position -1082 is important in the regulation of IL-10. Individuals homozygous for the *G* allele (*-1082GG*) have higher circulating IL-10, higher expression of IL-10 mRNA, and greater production of IL-10 after *in vitro* stimulation [20-22].

Results from studies of the *IL-10 -1082GA* polymorphism have been inconsistent and appear to depend on whether the study population and the controls are critically ill. In community-acquired pneumonia, the *-1082GG* genotype is associated with increased severity and risk of sepsis and increased mortality [23,24]. However, in studies on critical illnesses, the *-1082GG* genotype occurs less frequently among critically ill patients with organ failures than in healthy controls [25]. Among patients with established critical illnesses, the *-1082GG* genotype has been associated with significantly lower [26,27] or a trend to lower severity of illness, organ dysfunction and mortality [22,28].

The present study describes a nested case-control study of patients at risk for ARDS. The current authors investigated whether the *IL-10 -1082 GA* polymorphism was associated with susceptibility to developing ARDS and with mortality in ARDS.

MATERIALS AND METHODS

Study subjects

Details of the study population and design have been previously described [2]. Admissions to the intensive care units (ICUs) of the Massachusetts General Hospital (Boston, MA, USA) were screened daily for study-defined clinical risk factors for ARDS, such as sepsis, pneumonia, trauma, massive transfusion of eight or more units of blood within 24 h, or aspiration as defined previously [2]. Exclusion criteria included age <18 yrs, diffuse alveolar haemorrhage or chronic lung diseases and directive to withhold intubation. Patients with immunosuppression or treatment with granulocyte colony-stimulating factor were excluded. After November 2000, patients with immunosuppression from corticosteroid treatment were no longer excluded because of increasing use of steroids in sepsis.

Figure 1 displays the details of the study design and selection of cases and controls. ICU admissions with at least one defined risk factor for ARDS and no exclusion criteria were eligible for the prospective cohort and followed for development of ARDS, defined by respiratory failure requiring intubation and American European Consensus Committee (AECC) criteria, as previously described [2]. Those who fulfilled criteria for ARDS during hospitalisation were selected as cases in the case-control study. All patients who did not develop ARDS during hospitalisation and had no prior history of ARDS or prior enrolment

into the study were selected as controls. The Human Subjects Committees (Boston, MA, USA) approved the study, and informed written consent was obtained from all subjects or their appropriate surrogates.

Baseline clinical information was collected on admission to ICU. Vital signs and laboratory parameters from the first 24 h after ICU admission were collected for Acute Physiology, Age and Chronic Health Evaluation (APACHE) III study [29]. 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 [29]. Missing nonphysiology data, such as genotype failures, history of diabetes or tobacco use, were coded as missing.

ARDS cases were followed for all-cause 60-day mortality and daily multiple organ dysfunction score (MODS) for 28 days after the development of ARDS. MODS was defined and calculated according to criteria established in Brussels [30], whereby one point is given for each organ failure. Cardiovascular failure was defined as systolic blood pressure <90 mmHg or need for vasopressor. Renal failure was defined as creatinine >2.0 $mg \cdot L^{-1}$, hepatic failure by total bilirubin >2.0 $mg \cdot dL^{-1}$ and haematological failure as platelets <80,000$\cdot m^{-3}$. Respiratory failure was not included in the calculation of the organ dysfunction score in the ARDS patients.

Methods

Blood (10 mL) was collected for DNA extraction and PCR amplification. Genotyping was performed with the Sequenom MassARRAY system [31], with a random 5% of samples repeated. Laboratory personnel and research assistants were blinded to the case-control status or genotype of the subjects.

Analysis

Continuous data are presented as mean±SD or as median and the 25th to 75th percentile range, depending on the distribution. Univariate analysis was performed using Fisher's exact tests, ANOVA or Wilcoxon rank sum tests as appropriate. The Kaplan-Meier curves for 60-day ARDS survival were compared using the log-rank test. Variables with p-value of ≤0.2 on univariate analyses were studied in a backwards selection algorithm and eliminated if they did not meet a p-value of ≤0.1. Multivariate analyses consisted of logistic regression models for ARDS and Cox proportional hazard models for mortality in ARDS and included the following: 1) the gene effect; 2) results from backwards elimination; 3) significant interactions and clinically relevant parameters, such as septic shock; and 4) APACHE III scores for the development of ARDS [32]. Only four observations for the development of ARDS and one observation for mortality in ARDS were deleted from the final multivariate models due to missing values. A Hosmer-Lemeshow goodness-of-fit test was used to evaluate a logistic regression model fit. As per prior reports, individuals with the *-1082GG* genotype were compared with individuals with the *-1082A* allele (*-1082AA* and *-1082GA*) [22,24,27]. Effect modification was tested with an interaction term. ARDS patients lost to follow-up, discharged home alive prior to 60 days or survived to 60 days were censored at last contact. Only APACHE III score deviated from the proportional hazard assumption, as indicated by time-varying covariate (p=0.04), so the final model for ARDS survival was stratified by APACHE III score by quartiles. Daily MODS after development of ARDS was compared using mixed-effects models assuming an unstructured covariance matrix using the Proc Mixed procedure in a statistical software program. The fixed factors included the polymorphism and other potential confounders, such as age, trauma as a risk factor for ARDS, APACHE III scores, treatment with corticosteroids prior to admission, liver failure, transfusion, and septic shock. The number of days after the development of ARDS was considered to be a random factor. For patients discharged from the hospital within 28 days

of ARDS, their last available organ dysfunction score prior to 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. A p-value of 0.05 was considered statistically significant.

In the initial study design, assuming an α-error of 0.01 for multiple comparisons, 80% power and allele frequency of 0.51 for *-1082G*, a study with 560 cases and 1,120 controls would have a minimum detectable odds ratio (OR) for the development of ARDS of 1.43. Given the actual study size of 211 cases, 429 controls and α-error of 0.05 without adjusting for multiple comparisons, the minimum detectable OR would be 0.62 or 1.61 for ARDS and 0.45 or 2.2 for mortality in ARDS.

RESULTS

Patient population

The case-control study consisted of 237 ARDS cases and 477 controls recruited between September 9, 1999 and October 15, 2002 (fig. 1). A total of 18 (3%) patients failed genotyping. As 92% of the remaining patients were Caucasians, analyses were restricted to the 211 Caucasian cases and 429 Caucasian controls.

Clinical risk factors for ARDS and baseline characteristics on admission to the ICU are shown in tables 1 and 2. Variables in the final model for development of ARDS included direct pulmonary injury ($p<0.001$), trauma ($p<0.001$), age ($p<0.001$), female sex ($p=0.04$), diabetes ($p=0.001$), platelets $\leq 80{,}000 \cdot mm^{-1}$ ($p=0.002$), blood transfusion ($p<0.001$), septic shock ($p=0.2$) and APACHE III score ($p=0.4$). The Hosmer-Lemeshow statistic for the final model was 6.1 with eight degrees of freedom ($p=0.6$), indicating no significant lack of fit of the model.

Genotype analyses and development of ARDS

The allele frequency for the *-1082G* allele was 0.45. There was no discrepancy on repeat genotyping. Genotype frequency among cases and controls is shown in table 3. Among the controls, the genotype frequency deviated from that predicted by the Hardy-Weinberg equilibrium ($p=0.04$). Among all patients in the study, the APACHE III score on admission did not differ significantly by the *-1082GA* genotype ($p=0.8$; fig. 2a). The *-1082GG* genotype was associated with development of ARDS on the final multivariate analysis (crude OR 1.1; 95% confidence interval (CI; 0.74-1.6); adjusted OR 22; 95% CI (3.9-126)), but only in the presence of an interaction term between the *IL-10 -1082GG* genotype and age ($p<0.001$ for main effect and for interaction). Adjusting for any of the other covariates in the model changed the crude estimate <5% with no qualitative change in significance. This interaction between age and the *-1082GG* genotype was examined further after stratifying by age according to quartiles (table 3; fig. 3). The *-1082GG* genotype was significantly associated with an increased odds of developing ARDS among individuals <52 yrs of age (ORcrude 3.9; 95% CI (1.8-8.6); ORadj 5.1; 95% CI (2.0-13); $p<0.001$). However, when patients aged ≥52 yrs were examined together, the *-1082GG* genotype appeared protective against ARDS (ORcrude 0.70; 95% CI (0.44-1.1); OR_{adj} 0.59; 95% CI (0.35-0.99); $p=0.04$).

IL-10 -1082GA *polymorphism and outcomes in ARDS*

The 60-day mortality was 46% (98 out of 211) for ARDS cases. Clinical risks for ARDS and baseline characteristics between survivors and nonsurvivors of ARDS are shown in tables 1 and 2. Significant predictors in the final model for mortality in ARDS included age ($p<0.001$), APACHE III score ($p=0.001$), trauma ($p=0.08$), systolic blood pressure <90

mmHg (p=0.02), alcohol abuse (p=0.03) and blood transfusion (p=0.001). No significant interactions were found.

Compared with ARDS patients with *-1082AA* and *-1082GA* genotypes, ARDS patients with the *-1082GG* genotype had lower APACHE III scores on admission to the ICU (70.7±25.3 *versus* 81.0±23.2; p=0.008; fig. 2b) and higher ventilator-free days (median (interquartile range) 3 (0-12) versus 0 (0-10)), although the latter was not statistically significant (p=0.3). After development of ARDS, the *-1082GG* ARDS cases had lower daily organ dysfunction scores over time than the *-1082A* allele carriers (p=0.03; fig. 4). The 60-day mortality among the ARDS cases varied significantly with the *-1082GA* genotype (fig. 5). In comparison to the *-1082A* carriers, the *-1082GG* genotype was associated with decreased 60-day mortality in ARDS on crude analyses (hazard ratio (HR) 0.43; 95% CI (0.25-0.76); p=0.004) with the difference in mortality occurring early in the course of ARDS (fig. 5). On multivariate analysis after controlling for potential confounders and stratifying by APACHE III score on admission, the association between *-1082GG* genotype and ARDS mortality remained significant (HRadj 0.55; 95% CI (0.31-0.99); p<0.05).

DISCUSSION

In a study of ICU patients with clearly defined ARDS risk factors, the current authors found that the *-1082GG* genotype was associated with ARDS, but only in the presence of a significant interaction between the *-1082GG* genotype and age. Among patients with ARDS, significant associations were found between the *IL-10 -1082GG* genotype and lower severity of illness on presentation, lower daily organ dysfunction and lower mortality.

The present study has a number of strengths. First, the prospective determination of ARDS using the AECC definition helps minimise phenotype misclassification. Secondly, clearly defined at-risk controls were used. Using critically ill controls who have the opportunity to develop the outcome is more clinically relevant than using healthy individuals. This also reduces any confounding from any possible association between the gene and the risk condition, such as sepsis or pneumonia.

The significance of the interaction between age and the *-1082GG* genotype on the risk of ARDS is unclear. It is possible that the relative pro- *versus* anti-inflammatory response to infection and injury differs with age. Elderly patients have elevated levels of circulating pro-inflammatory cytokines, such as IL-6 and TNF-α at baseline and in sepsis [32-34]. In a population prone to a higher inflammatory state, a genotype associated with high anti-inflammatory response may be protective against development of ARDS, whereas in another group less prone to inflammation, the effect may be different. However, given that the baseline inflammatory state of the patients could not be examined here, this finding should be used for hypothesis generation. Additional studies will be needed to define the biological interaction between the *-1082GG* genotype and age in ARDS.

The current finding that the high IL-10-producing *-1082GG* genotype is protective against mortality and organ failure in ARDS is consistent with the role of intense inflammation in acute lung injury. Other studies have found significant or nonsignificant associations between the *IL-10 -1082G* allele and decreased organ failure, severity of illness or mortality in critically ill patients [22,26-28]. However, in noncritically ill patients with pneumonia, the *-1082GG* genotype was associated with increased severity of sepsis and mortality [23,24]. The different patient populations studied may explain this discrepancy. It is increasingly recognised that the overall balance between pro-inflammatory and anti-inflammatory responses is important in the response to injury and in ARDS [35-37]. In patients with pneumonia, a genotype associated with high IL-10 production may lead to more

immunosuppression, resulting in more severe disease burden and outcomes. In contrast, the present findings were in ARDS patients. As intense inflammation is a key feature in the pathogenesis of ARDS, the present authors may have selected for patients with a high pro-inflammatory condition. In such cases, a genotype associated with high IL-10 may have a beneficial modulating effect. Indeed, other studies that have found a protective effect of the *-1082G* allele were carried out in critically ill patients or in meningococcaemia in which an intense pro-inflammatory response is well recognised [26,27]. Given the high frequency of the *-1082G* allele in the population, it is reasonable that the high IL-10-producing *-1082GG* genotype may not be universally detrimental. Thus, it is important to note that the current findings may not be applicable to non-ARDS patients.

It is also interesting to note that the mortality difference among ARDS patients with the *-1082GG* genotype occurs early in the course of ARDS, reaching a plateau in the later course of ARDS. The potential benefit of a high anti-inflammatory genotype may not be the same through all stages of ARDS. Certainly the cause of mortality and the balance between pro- and anti-inflammatory responses in critical illnesses differs over time [38-40].

Among the controls in the present study, the genotype frequency of the *-1082GA* polymorphism deviated from that predicted by the Hardy-Weinberg equilibrium. This is unlikely to be due to genotyping error as repeat genotyping in a random subset of patients revealed no discrepancy. However, since the controls were critically ill patients with organ failure, it is possible that deviation from Hardy-Weinberg occurred because of a possible association between the *-1082GA* polymorphism and the critical illness requiring ICU admission, as has been reported by other groups [25,41].

The association between the *-1082GG* genotype, lower APACHE III score and lower ARDS mortality is unlikely to be due to the increased odds of younger patients with this genotype to develop ARDS. The association between the *-1082GG* genotype and APACHE score in ARDS was still significant even after age was removed from the score ($p<0.03$). The association between the *-1082GG* genotype and decreased mortality remained even after restricting the analyses to those patients <52 yrs of age (HR 0.083; 95% CI (0.01-0.68); $p=0.006$; data not shown).

The association found between the *IL-10 -1082GA* polymorphism and ARDS mortality is unlikely to be due to type I error. The polymorphism was chosen *a priori* based upon previous studies supporting its role in critical illness. The results are consistent with previous reports on organ failure and mortality in critical illness. Additionally, the results are supported by results of secondary outcomes, such as severity of illness on admission and organ failure after development of ARDS. Nevertheless, as is true of all genetic association studies, the current findings will need to be confirmed in other populations.

The present authors acknowledge that there are some limitations to the study. Only one polymorphism was examined in the *IL-10* gene and *-1082GA* polymorphism is known to be linked to other functional polymorphisms on the *IL-10* gene in other disease conditions [42-44]. Thus, the possibility that the association found in the current study is due to linkage disequilibrium between the *-1082G* allele and the disease locus cannot be excluded. A haplotype approach would be better to examine this possibility. In addition, the functional significance of the *-1082GA* polymorphism cannot be confirmed in the current study. Due to the study design, the results may not be generalisable to the community setting, immunocompromised hosts or healthy 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 [45], but it does not permit extrapolation of the results to other ethnic groups.

In conclusion, the present study reports an interaction between the *interleukin-10 -1082GG* genotype and age on the development of acute respiratory distress syndrome. Among cases of acute respiratory distress syndrome, the *interleukin-10 -1082GG* genotype was associated with decreased severity of illness, decreased organ dysfunction and decreased mortality in acute respiratory distress syndrome. Additional studies are needed to confirm these findings in other populations with other risk factors.

Acknowledgments

The authors would like to thank W-L. Zhang, K. McCoy, T. McCabe, C. Schwartzenburg, B. Bean and L. Lineback for patient recruitment; A. Solomon, A. Shafer and L. Shimada for research support; L. Su for laboratory expertise; J. Frelich, M. Chertok, S. Mucci, R. Rivera for data management; and P.D. Boyce for assistance in radiological interpretation.

SUPPORT STATEMENT

The following work was supported by research grant RO1 HL60710 from the National Heart, Lung and Blood Institute (NHLBI; Bethesda, MD, USA). M.N. Gong was supported by grant K23 HL67197 from NHLBI and M.Z. Wang by grant T32 ES07069 from the National Institute of Health (Bethesda, MD, USA).

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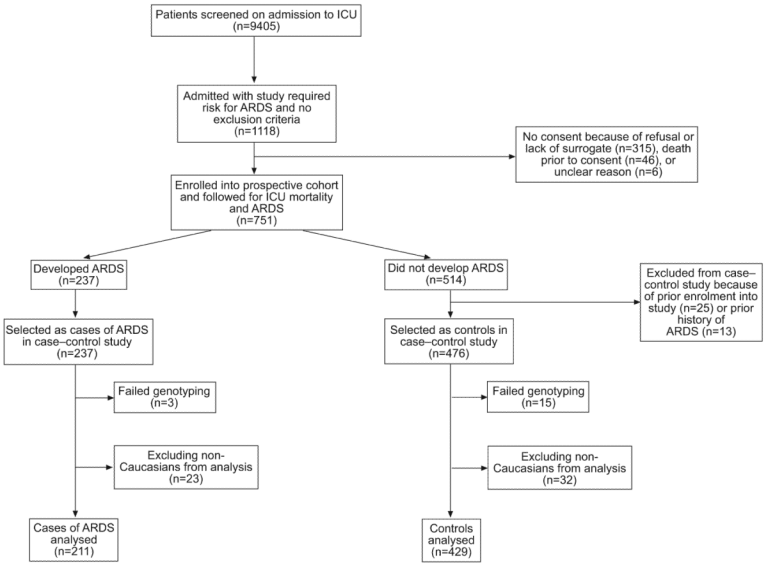

FIGURE 1.

Flow diagram of study design and patient selection for the case-control study. ICU: intensive care unit; ARDS: acute respiratory distress syndrome.

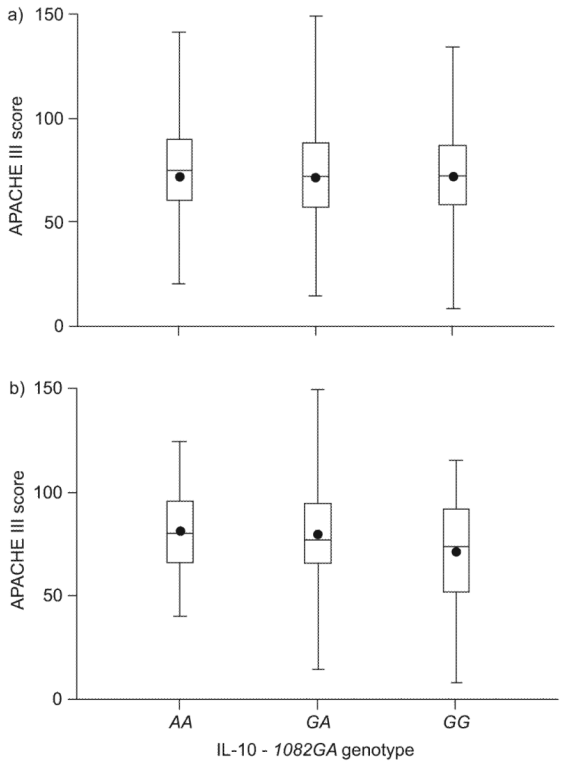

FIGURE 2.

Acute Physiology, Age and Chronic Health Evaluation (APACHE) III score on admission to the intensive care unit by genotype for the interleukin (IL)-10 -*1082GA* polymorphism among a) 640 cases and controls (*AA*: n=200; *GA*: n=292; *GG*: n=148; p=0.8) and b) the 211 ARDS cases (*AA*: n=58; *GA*: n=102; *GG*: n=51; p=0.008). The box denotes the interquartile range (25-75%) and the horizontal line indicates the median. Error bars `indicate the 95% confidence intervals. •: mean.

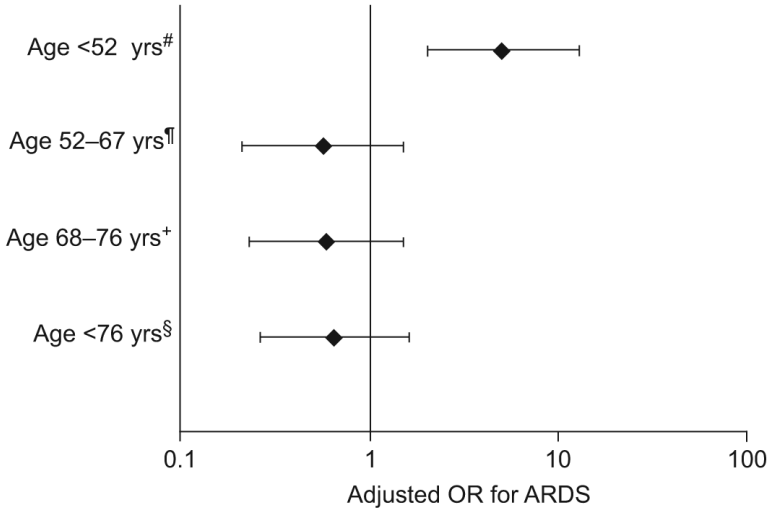

FIGURE 3.

Odds ratio (OR) for the development of acute respiratory distress syndrome (ARDS) among individuals homozygous for *-1082G* allele (*1082GG*) compared with carriers of the *-1082A* allele after stratifying by age quartiles. OR were adjusted for direct pulmonary injury, septic shock, trauma, female sex, haematological failure (platelet <80,000·mm^{-1}), transfusion of red cells and Acute Physiology, Age and Chronic Health Evaluation III (without the arterial oxygen tension/inspiratory oxygen fraction component). There was significant interaction by age on the association between the interleukin-10 *-1082GG* genotype and development of ARDS ($p<0.001$). #: n=161; ¶: n=165; +: n=155; §: n=154.

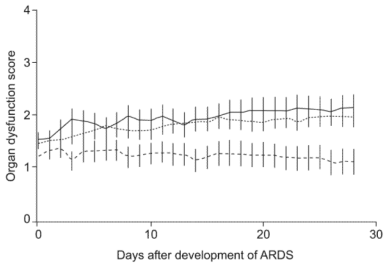

FIGURE 4.

Daily Brussels multiple organ dysfunction score for 28 days after development of acute respiratory distress syndrome (ARDS) according to interleukin-10 -*1082GA* genotype (···) for the 211 ARDS cases. In comparison with the -*1082A* carriers (-*1082AA* (—) and -*1082GA* (- - -)), ARDS patients with the -*1082GG* genotype had less organ failure over the course of their ARDS (p=0.03).

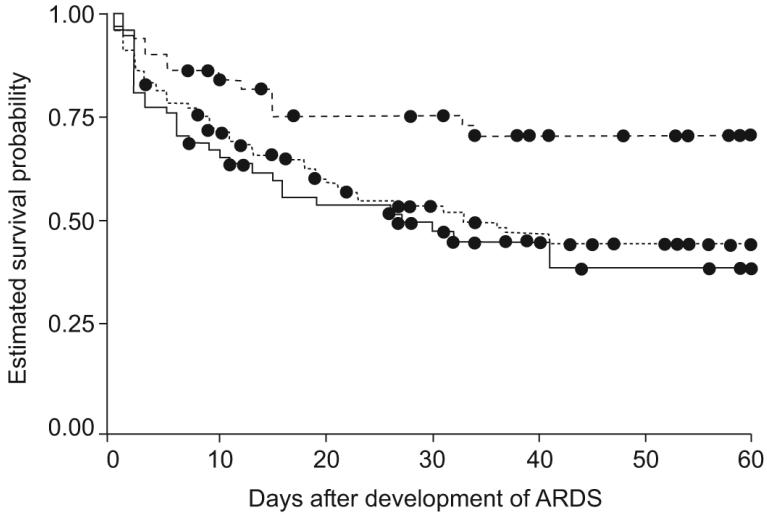

FIGURE 5.

Kaplan-Meier 60-day survival curves in acute respiratory distress syndrome (ARDS) according to the interleukin-10 *-1082GA* genotype. Compared with *-1082AA* (—) and *-1082GA* (···) genotypes. ARDS patients with the *-1082GG* genotype (- - -) had better survival (p=0.004). •: censored.

TABLE 1

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

Risk for ARDS[#]	Development of ARDS			Mortality in ARDS		
	Controls[¶]	Cases[+]	p-value	Survivors[§]	Nonsurvivors[*f*]	p-value
Sepsis syndrome	163 (38)	67 (32)	0.1	44 (39)	23 (23)	0.02
Pneumonia source	85 (20)	52 (25)	<0.001	33 (29)	19 (19)	0.6
Extrapulmonary source	78 (18)	15 (7)	<0.001	11 (10)	4 (4)	
Septic shock	191 (45)	114 (54)	0.03	51 (45)	63 (64)	0.006
Pneumonia source	85 (20)	78 (37)	<0.001	35 (31)	43 (44)	>0.9
Extrapulmonary source	106 (25)	36 (17)	<0.001	16 (14)	20 (20)	
Trauma	38 (9.0)	9 (4)	0.04	8 (7)	1 (1)	0.04
Multiple transfusions	50 (12)	26 (12)	0.8	13 (12)	13 (13)	0.8
Aspiration	32 (7)	24 (11)	0.1	13 (12)	11 (11)	>0.9
One or more risk for ARDS	48 (11)	29 (14)	0.3	16 (14)	13 (13)	>0.9
Direct pulmonary injury[##]	204 (48)	143 (68)	<0.001	78 (69)	65 (66)	0.8
Indirect pulmonary injury[¶¶]	225 (52)	68 (32)	<0.001	35 (31)	33 (34)	

Data are presented as n (%), unless otherwise stated.

[#] numbers of controls and cases with each risk add up to >640 patients because of multiple risks in 75 patients

[¶] n=429

[+] n=211

[§] n=113

[*f*] n=98

[##] pneumonia, aspiration or pulmonary contusions were categorised as direct pulmonary injury

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

TABLE 2

Baseline characteristics between cases of acute respiratory distress syndrome (ARDS) and controls and survivors and nonsurvivors in ARDS

	Development of ARDS			Mortality in ARDS		
	Controls[#]	Cases[¶]	p-value	Survivors[+]	Nonsurvivors[§]	p-value
Females	172 (40)	101 (48)	0.07	50 (44)	51 (52)	0.3
Age yrs	69 (18-94)	65 (18-97)	0.04	57 (18-89)	73 (22-97)	<0.001
APACHE III *f*	64 (14-130)	68 (8-136)	0.09	69 (8-115)	88 (29-150)	<0.001
Diabetes ##	114 (27)	33 (16)	0.002	19 (17)	14 (14)	0.7
History of alcohol abuse	42 (10)	26 (12)	0.3	9 (8)	17 (17)	0.06
Tobacco abuse ¶¶	211 (49)	104 (49)	0.4	56 (50)	48 (49)	0.9
Chronic liver disease ##	18 (4)	12 (6)	0.7	4 (4)	8 (8)	0.2
End-stage renal disease	22 (5)	6 (3)	0.2	2 (2)	4 (4)	0.4
History of steroid use	37 (9)	20 (9)	0.7	6 (5)	14 (14)	0.03
Transfusion of PRBC	213 (50)	133 (63)	0.001	64 (57)	69 (70)	0.03
Number of PRBC transfused	0 (0-74)	2 (0-63)	0.005	1 (0-31)	2 (0-63)	0.02
Systolic BP <90 mmHg	295 (69)	162 (77)	0.04	84 (74)	78 (80)	0.4
Creatinine >2.0 mg·L^{-1}	146 (34)	65 (31)	0.4	28 (25)	37 (38)	0.05
Bilirubin >2.0 mg·dL^{-1}	52 (12)	39 (18)	0.04	14 (12)	25 (26)	0.02
Haematological failure platelets ≤80000·mm^{-1}	58 (14)	47 (22)	0.006	20 (18)	27 (28)	0.1

Data are presented as n (%) or median (range), unless otherwise stated. APACHE: Acute Physiology, Age and Chronic Health Evaluation; PRBC: packed red blood cells; BP: blood pressure.

[#] n=429

[¶] n=211

[+] n=113

[§] n=98

f for development of ARDS, APACHE III physiology score for cases and controls were calculated without the arterial oxygen tension/inspiratory oxygen fraction component; for survivors and nonsurvivors in ARDS, the APACHE III physiology score was calculated with all components

chronic health information was missing in one case and two controls

¶¶ tobacco history was missing in 56 (26%) cases and 97 (22%) controls.

TABLE 3

Genotype frequency of the interleukin (IL)-10 -*1082GA* polymorphism among cases and controls after categorising by quartiles in age

Age yrs	Subjects n	Cases of ARDS	Cases of ARDS by IL-10 -*1082GA* genotype			
			-1082AA	*-1082GA*	*-1082GG*	p-value
All ages	640	211 (33)	58 (29)	102 (35)	51 (34)	0.4
<52	162	63 (39)	17 (30)	24 (33)	22 (65)	0.003
52-67	167	59 (35)	14 (32)	34 (40)	11 (29)	0.5
68-76	156	46 (29)	15 (29)	23 (32)	8 (24)	0.7
<76	155	43 (28)	12 (24)	21 (33)	10 (24)	0.5

Data are presented as n or n (%), unless otherwise stated. ARDS: acute respiratory distress syndrome.