Pharmacogenomic influence on sepsis outcome in critically ill patients

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SUMMARY

In infectious and inflammatory diseases, pharmacogenetics affects treatment efficacy and toxicity. Moreover, recent studies suggest its important role in predicting the clinical outcome of sepsis. Our aim was to investigate the influence of single nucleotide polymorphisms (SNPs) in genes which we supposed to be involved in linezolid elimination upon sepsis outcome. Fourteen ICU-admitted patients in therapy with intravenous linezolid (600 mg q12 h) were enrolled and classified into three groups: group 0 for sepsis, 1 for severe sepsis and 2 for septic shock. Genotyping for SNPs in MDR1 3435 rs1045642 C>T, 2677 rs2032582 G>T and 1236 rs1128503 C>T, MRP2 -24 rs717620 G>A and 1249 rs2273697 G>A, MRP4 *879 rs1059751 T>C and 3348 rs1751034 T>C, BCRP1 421 rs2231142 C>A and 1194+928 rs13120400 T>C, -127 rs4149170 G>A and OCT1 480 rs683369 C>G genes was done using real-time PCR allelic discrimination assay. The Mann-Whitney statistical test was used to analyse variables. MDR1 2677 (p=0.012), MRP2 1249 (p=0.038), MRP4 *879 (p=0.032) and 3348 SNPs significantly influenced the sepsis score. Our study, despite its limited sample size, could be decisive for early sepsis prediction and may improve the management of critically ill patients.

Keywords: ICU, MDR1 2677, MRP2 1249, MRP4 *879, MRP4 3348.

INTRODUCTION

Sepsis is a multifactorial complex syndrome with many non-specific symptoms, which remains a difficult challenge among patients in Intensive Care Units (ICUs). Despite treatment and prevention advances, sepsis incidence is rising, with a mortality rate around 20-30% [1].
In infectious and inflammatory diseases, individual genomic variability affects clinical outcome, incidence and severity. Rautanen et al. performed a large-scale genome-wide study in a well-defined group of ICU admitted patients with sepsis caused by pneumonia; they found a significant association between proto-oncogene tyrosine-protein kinase (*FER*) gene variant (*rs4957796*) and 28-day survival [2].
Another study by Russell et al. revealed the role of protein C gene (*PROC*) *rs2069912 T* allele in mortality 50% to 30% reduction, in sepsis patients treated with Xigris® [3]. Moreover, in 2013, Paludo et al. observed a significant higher frequency of septic shock, considering ICU hospitalized, with manganese superoxide dismutase (*SOD2*) *rs4880 C* allele [4].
In this study, we investigated the possible relationship between different degree of sepsis and single nucleotide polymorphisms (SNPs) in MDR1, MRP2, MRP4, BCRP1, OAT1 and OCT1 genes.
MDR1 (multidrug resistant protein 1) gene encodes the efflux pump P-glycoprotein, a transporter expressed also in the luminal site of kidney

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tubular cells, where it contributes to the active secretion of drugs. It is involved in linezolid, tobramycin, minocycline, riluzole, azithromycin, daptomycin, rifampicin, grepafloxacin, levofloxacin and sparfloxacin elimination [5]. MRP2 (multidrug resistance protein 2) gene is localized in the apical membrane of polarized cells, thus renal ones, mediating excretion. It plays a role in the secretion of levofloxacin, ceftriaxone, cefoperazone, cefbuperazone and cefpiramide [6]. MRP4 (multidrug resistance protein 4) gene encodes different compounds transport protein, located in many tissues, as well as in kidney; for example, it acts in ciprofloxacin intracellular excretion [7]. Breast cancer resistance protein (BCRP1, also known as ABCG2) may influence drug toxicity; this ATP-binding cassette transporter is expressed in gastrointestinal tract and liver and it is involved in absorption, distribution and excretion of a wide variety of drugs. Germline polymorphisms in this gene have been described as affecting expression, cellular localization and substrate recognition of the encoded protein; more than 24 variations have been reported [8-10]. The prototypical organic anion transporter OAT1 acts as an organic anion transporter or organic cation transporter and it is responsible for the transport of many small water-soluble organic anion drugs, toxins, metabolites, and signaling molecules [11]. OCT1 was the first member of the organic cation transporter family cloned; it is primarily expressed in liver and to a lesser extent in other organs. The protein is localized to the basolateral membrane of centrilobular hepatocytes, proximal tubule cells, Sertoli cells, enterocytes, and in serotoninergic neurons of the small intestine. Prominent expression of OCT1 on the sinusoidal membrane of hepatocytes suggests that this transporter mediates the excretion of cationic drugs [7].

MATERIALS AND METHODS

Patients treated with intravenous linezolid, admitted to ICUs at City of Science and Health, Molinette Hospital, Turin (Italy), were enrolled. Inclusion criteria were: age above 18 years, no HIV/HCV/HBV infection and no concomitant treatment with substrates, inhibitors or inductors of MDR1, MRP4, MRP2, BCRP1, OAT1 and/or OCT1 enzymes.

Ethical approval was given by the Ethical Committee (ASPIRE Project 2010); a written informed consent was obtained from each enrolled subject.
Patients were diagnosed with sepsis, severe sepsis and septic shock according to the American College of Chest Physicians/Society of Critical Care Consensus Conference definition [12].
A venous blood sample was obtained from each patient (3 mL EDTA); whole blood was stored at -80°C and DNA extracted using the QIamp DNA Mini Kit (Qiagen, Valencia, CA). DNA was used for the real-time PCR (BIORAD, Milano, Italia) reaction.
The allelic discrimination analysis was performed using the TaqMan assays (Applied Biosystems, Foster City, CA).
Analyzed SNPs were MDR1 3435 rs1045642 C>T, 2677 rs2032582 G>T and 1236 rs1128503 C>T, MRP2 -24 rs717620 G>A and 1249 rs2273697 G>A, MRP4 *879 rs1059751 T>C and 3348 rs1751034 T>C, BCRP1 421 rs2231142 C>A and 1194+928 rs13120400 T>C, OAT1 -127 rs4149170 G>A and OCT1 480 rs683369 C>G.
Continuous and non-normal variables were summarized as median values and the interquartile range (IQR), 25th to 75th percentiles, was calculated to measure the statistical dispersion of the data. All the variables were tested for normality with the Shapiro-Wilk test. The correspondence of each parameter was evaluated with a normal or non-normal distribution, through Kolmogorov-Smirnov test. All the SNPs were tested for Hardy-Weinberg equilibrium (χ^2 Test). Linkage Disequilibrium (LD) was evaluated with Haploview 4.2 software (Cambridge, Massachusetts, USA). Kruskal-Wallis and Mann-Whitney tests have been used to compare sepsis stage and SNPs, considering the level of statistical significance (p-value <0.05). All the statistic tests were performed with IBM SPSS Statistics 22.0 for Windows (Chicago, Illinois, USA).

RESULTS

Fourteen Caucasian patients (9 males and 5 females) were enrolled: 5 (36%) had sepsis, 2 had severe sepsis (14%) and 7 (50%) had septic shock. Patients with sepsis, severe sepsis and septic shock were given a score equal to 0, 1 and 2, respectively. Median age and median body mass

index were 65.9 years (IQR 46.4-75.4 years) and 24.00 Kg/m^{-2} (IQR, 23.25-26.00 Kg/m^{-2}), respectively. Our cohort demographical and pharmacokinetic characteristics were resumed in Table 1.

Pharmacogenetic analyses were successful for all the patients and no controversial results from duplicate analyses were found. For all of the analysed loci, the Hardy-Weinberg equilibrium was demonstrated and their MAF values were superimposable with those published. Only MDR1 2677 rs2032582 G>T and 1236 rs1128503 C>T SNPs resulted in complete LD (D'=1) in our population.

MDR1 2677, MRP2 1249, MRP4 *879 and 3348 SNPs significantly influenced sepsis score (Figure 1). We observed a significant association between MDR1 2677 TT genotype and severe sepsis (score 2) and between GG/GT group and sepsis (score 0) (p=0.012; Figure 1A).

Regarding MRP2 1249 G>A SNP, we showed a significant different distribution between 0 sepsis score and GG genotype and of 1 and GA genotype (p= 0.038; Figure 1B); AA patients for this SNP were absent in our cohort.

Considering MRP4 gene, patients with sepsis score 1 and 2 had all at least one T allele for *879 variant (p=0.032; Figure 1C) and no patients with 0 and 2 sepsis scores had 3348 CC genotype (p=0.040; Figure 1D).

DISCUSSION AND CONCLUSIONS

It was already known the important role of pharmacogenetic in clinical outcome of sepsis patients; it is related to the different ability of each subject to metabolize drugs depending on the genetic predisposition; moreover, pharmacogenetic could explain some interindividual variability, considering inheritance as a determinant of drug response [13, 14]. Thus, we evaluated the effect of SNPs in MDR1, MRP2, MRP4, BCRP1, OAT1 and OCT1 genes on sepsis score in patients treated with linezolid. We observed the role of MDR1 2677, MRP2 1249, MRP4 *879 and 3348 SNPs on sepsis stage.

Recently, it has been reported that MDR1 2677 TT genotype has been associated with daptomycin higher plasma levels and reduced clearance [5]. Here, we found that patients with at TT mutant homozygous genotype had 1 and 2 of sepsis score, probably due to a higher elimination of the drug, thus a reduced treatment effect.

Concerning MRP2 1249 SNP, which influences deferasirox pharmacokinetic, we observed 1 and 2 sepsis stage patients only in the GG genotype group; this probably suggests a lower linezolid levels in the organism, resulting in a minor effect of the drug [6].

Table 1 - Demographical and clinical characteristics of patients.

Patient	*Age (Years)*	*Gender (F: female; M: male)*	*BMI (Kg/m^2)*	*Diabetes (Yes=1; No=0)*	*Cystic fibrosis (Yes=1; No=0)*	*Linezolid dose (mg q12 h)*	*Duration of linezolid therapy (days)*	*Endocarditis (Yes=1; No=0)*	*Sepsis stage**	*Creatinine serum levels (mg/dL)*
A	33,4	F	21,0	0	0	600	12	0	2	2,00
B	74,5	M	24,0	0	0	600	15	0	2	1,20
C	44,1	M	26,0	1	0	600	18	0	2	1,40
A	53,1	M	24,0	0	0	600	7	0	2	0,86
B	63,9	M	33,0	0	0	600	4	0	0	1,11
C	77,3	F	24,0	0	0	600	13	0	0	1,91
A	25,8	F	18,0	0	1	600	10	0	0	0,32
B	72,0	M	24,0	0	0	600	8	0	2	2,80
C	74,0	M	26,0	0	0	600	9	0	2	1,70
A	62,1	M	23,0	0	0	600	15	0	0	3,28
B	79,0	M	28,0	0	0	600	5	0	0	1,98
C	83,7	M	28,0	1	0	600	12	0	1	1,00
A	23,7	F	25,0	0	0	600	8	1	2	0,46
B	65,9	F	20,0	1	0	600	14	0	1	3,20

BMI: Body Mass Index; *sepsis stages were 0=sepsis, 1=severe sepsis and 2=septic shock.

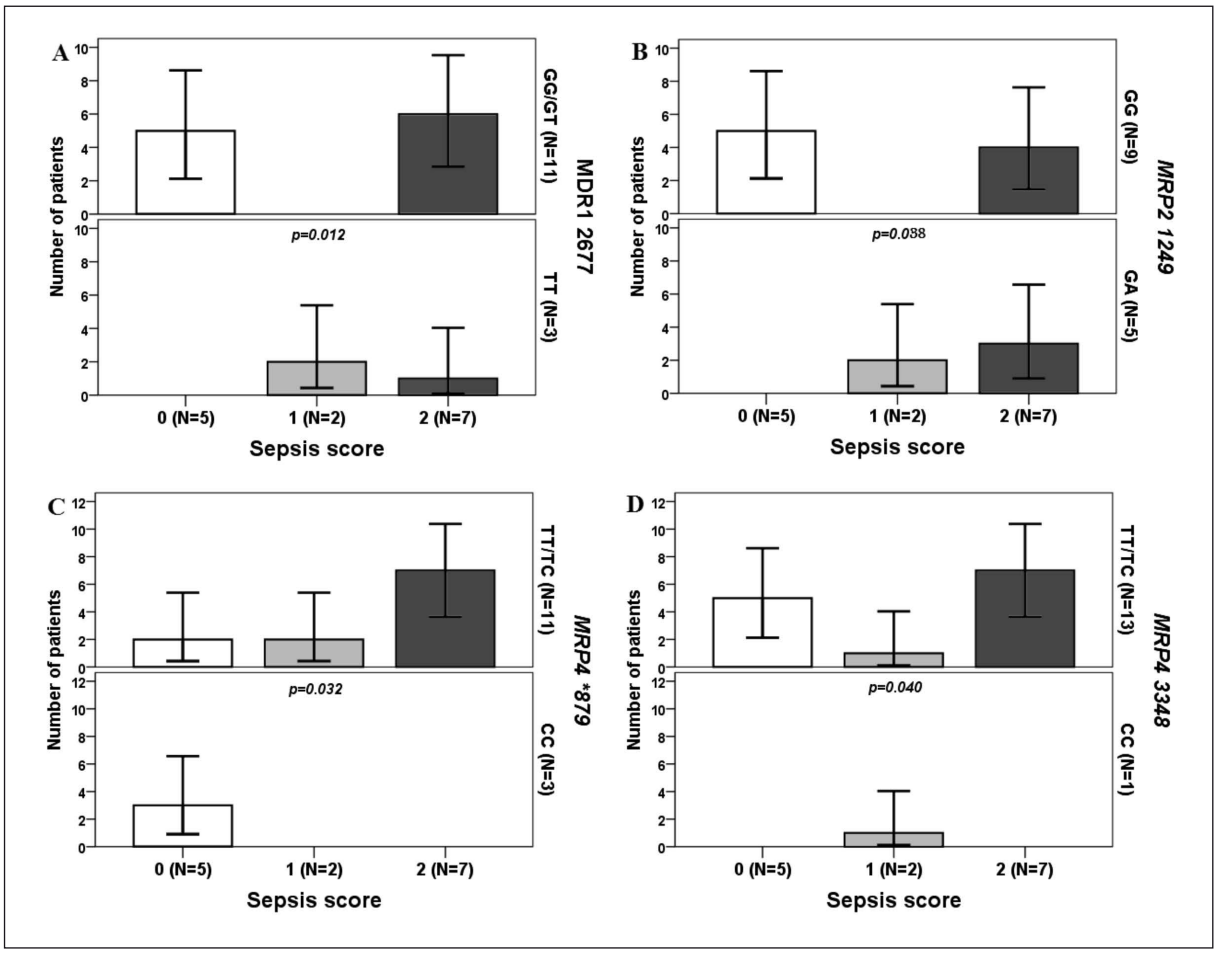

Figure 1 - Frequency plots of MDR1 2677, MRP2 1249, MRP4 *879 and MRP4 3348 SNPs divided by sepsis score. Distribution of genotypes by sepsis score was depicted; the frequency plot is displayed on the right as a bar chart with vertical grouping by the row variable genotype groups. Black lines in boxes represent 95% interval of confidence. The chart bar displayed at the bottom showed the three different sepsis scores:
- 0 for sepsis, light grey, 5 patients;
- 1 for severe sepsis, grey, 2 patients;
- 2 for septic shock, dark grey, 7 patients.

Hypothesis tests on contingency tables based on Chi-square have been used to compare sepsis score between different genotypes ($p<0.05$); each showed p was referred to the entire panel.
A. Frequency plots of MDR1 2677 GG/GT (N=11) and TT (N=3) genotype groups divided by sepsis score; p=0.012.
B. Frequency plots of MRP2 1249 GG (N=8) and GA (N=5) genotype groups divided by sepsis score; p=0.038.
C. Frequency plots of MRP4 *879 TT/TC (N=11) and CC (N=3) genotype groups divided by sepsis score; p=0.032.
D. Frequency plots of MRP4 3348 TT/TC (N=13) and CC (N=1) genotype groups divided by sepsis score; p=0.040.

Considering MRP4 *897 and 3348 SNPs, to date in literature are not reported pharmacogenomic evidences.

In this study, it was shown an influence of *897 T allele on severe sepsis and septic shock prediction, thus a probable treatment inefficacy. Instead, for the variant 3348, despite the statistical significance, there was no a good distribution of the analyzed groups.

Based on obtained results, we supposed that probably only MRP4 *879 SNP was representative in our population (Figure 1C).

The limited number of enrolled patients is explained by our choice to reduce bias, due to

different ethnicities and concomitant therapies administration, which could alter the obtained results. Notwithstanding the small study sample, these preliminary findings could contribute to explain the high intervariability in sepsis susceptibility. Moreover, this study underlines the need to improve sepsis diagnostic and treatment actions: patient genetic profile could be useful for sepsis early prediction and may enhance critically ill patients' management. In conclusion, these pharmacogenetic data are the first obtained and suggest the possible usefulness of genetic-based sepsis stage prediction, despite the confounding factors associated to ICU patients. More detailed studies, in bigger and different cohorts are required to confirm our results; moreover, the used of revised sepsis definition to divided the population, could reveal more details [15].

Conflict of interest. The authors have no conflicts of interest to disclose.

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