

Post-irradiation Hyperamylasemia Is a Prognostic Marker for Allogeneic Hematopoietic Stem Cell Transplantation Outcomes in Pediatric Population: a Retrospective Single-centre Cohort Analysis.

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Research

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Abstract

Background: Total body irradiation (TBI) is a mandatory step for patients with acute lymphoblastic leukemia (ALL) undergoing allogeneic hematopoietic stem cell transplantation (HSCT). In the past, amylases have been reported to be a possible sign of TBI toxicity. We investigated the relationship between total amylases (TA) and transplant-related outcomes in pediatric recipients.

Methods: We retrospectively analyzed the medical records of all the patients who underwent allogeneic HSCT between January 2000 and November 2019. Inclusion criteria were the following: recipient's between 2 and 18, diagnosis of ALL, no previous transplantation, and use of TBI-based conditioning. Serum total amylase and pancreatic amylase were evaluated before, during and after transplantation. Cytokines and chemokines assays were retrospectively performed.

Results: 78 patients fulfilled the inclusion criteria. 57 patients were treated with fractionated TBI and 21 with a single dose regimen. Overall survival (OS) was 62.8%. Elevated values of TA were detected in 71 patients (91%). TA were excellent in predicting the OS (AUC = 0.773; 95% CI = 0.66-0.86; $P < 0.001$). TA values below 374 U/L were correlated with a higher OS. The highest mean TA values (673 U/L) were associated with a high disease-progression mortality rate. TA showed high predictive performance for disease progression-related death (AUC = 0.865; 95% CI = 0.77 – 0.93; $P < 0.0001$). Elevated TA values were also connected with significantly higher levels of proinflammatory cytokines such as TNF- α , IL-6 and RANTES ($P < 0.001$).

Conclusions: This study shows that TA is a valuable predictor of post-transplant OS and increased risk of leukemia relapse.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a highly specialized medical procedure that, nearly 60 years ago, introduced the first regenerative approach to clinical practice [1, 2]. Although HSCT technology had evolved considerably in recent years, total body irradiation (TBI) remained one of the leading conditioning regimens in pediatric and adult patients with acute lymphoblastic leukemia (ALL) [3, 4].

In the late 1970s, the TBI procedure underwent a radical change, passing from single high-dose administrations to fractionated regimens [5], remaining substantially unchanged to the present, although methodological differences still exist between centres and countries [6].

TBI has significant advantages over high-dose chemotherapy, including accessibility to “sanctuaries” sites such as the testicles and central nervous system, homogeneity of high doses throughout the body, absence of concerns for drug excretion or detoxification, and cross-resistance in combination with chemotherapy, as well as the capacity to shield or boost sites of interest [6].

However, TBI is responsible for many significant side effects such as veno-occlusive disease (VOD), renal toxicity, interstitial pneumonitis, secondary malignancies, reproductive insufficiency, as well as growth retardation [7]. Their incidence has dramatically reduced after introducing fractionated regimens and decreasing dose rates [8–10].

In recent years, a great deal of effort has been made to reduce TBI conditioning's toxicity and identify people at higher risk of developing complications, aiming for personalized radiotherapy [11–13], including the identification of molecular biomarkers which might predict the response and tolerability of this procedure [11, 14]. Increased amylase levels, both pancreatic and salivary, and clinical manifestations of acute parotitis or pancreatitis, have been reported during TBI-based conditioning [15–17]. We decided to investigate the possible relationship between TA values and our cohort of patients' transplant outcomes.

Materials And Methods

Study population

A retrospective single-centre cohort analysis was conducted at the Pediatric Bone Marrow Transplant Unit of the Institute for Maternal and Child Health "IRCCS Burlo Garofolo" in Trieste, Italy. The Institutional Review Board of the institute (reference IRB-BURLO no. 03/2020) approved the study protocol. The parents of all subjects enrolled signed written consent for the collection and use of their personal data. Medical records of 226 patients who underwent allogeneic HSCT between January 2000 and November 2019 were investigated.

Inclusion and exclusion criteria

Inclusion criteria were the following: subjects between 2 and 18 years of age at the time of HSCT, diagnosis of ALL, no history of previous transplants, myeloablative conditioning regime including high dose TBI, documented serum TA, and pancreatic α -amylase levels before and during TBI-based conditioning. Patients were excluded if they had documented abnormal TA and pancreatic α -amylase levels one month before HSCT.

Study predictors

We collected data on the overall survival (OS) and the cumulative incidence of death. We gathered data on early (100 days) post-transplant complications such as TBI-related organ toxicity, infections, neutrophil and platelets engraftment, and acute graft-versus-host disease (GVHD). We also collected data on late complications, including chronic organ injury such as chronic GVHD, hypothyroidism, pancreatic insufficiency, cataract, and growth hormone deficiency. The causes of death resulted in the leukemia relapse and transplant-related mortality (TRM). GVHD, systemic infection, and organ toxicity were included in the type of outcomes defined as TRM.

HSCT procedure and TBI treatment

All 78 pediatric patients affected by ALL received high-dose TBI-based standard myeloablative conditioning for an allogeneic HSCT. In patients under the age of 2, TBI was omitted, according to the national protocol for ALL of the Italian Association of Pediatric Hematology and Oncology (AIEOP). We defined two TBI protocol groups: the first received a standard dose of 12 Gy, delivered in 6 fractions; the second one received 7.5 Gy in a single dose. A linear, accelerator-based, latero-lateral irradiation was employed for both the procedures. Plexiglass slabs were used to compensate for missing tissue in the head and neck, lead tablet in the lower leg regions, and lung shielding with the lateral position's upper limbs. In vivo dosimetry was performed with thermoluminescence dosimetry (TLD) only until 2003. Subsequently, the double-check of delivered dose and dose homogeneity was performed with Gafchromic EBT3 film and MOSFET (Metal Oxide Semiconductor Field Effect Transistor) detectors.

The conditioning regimen and GVHD prophylaxis were conducted as previously described [18].

Total amylase and pancreatic α -amylase values assessment

Serum TA was evaluated before, during, and after the TBI treatment, until its normalization. Two TA assessments were considered mandatory: the first one at baseline, before starting the conditioning, the second one either after the first day of TBI or before the third TBI session. With the total amylase assessment, we analyzed the pancreatic α -amylase values to prove that the post-TBI total amylase raise was caused by an increase of the α -amylase only. Amylase levels were expressed in unity per liter (U/L). Total amylase levels above 100 U/L were considered abnormal.

Assessing TBI-related inflammatory status

The analysis of 27 cytokines and chemokines, namely IL-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN- γ , IP-10, MCP-1(MCAF), MIP-1 α , PDGF-bb, MIP-1 β , RANTES, TNF- α , VEGF was carried out on plasma samples with multiple immunoassays, using a bead-based magnetic sensor (27 human-Bio-Plex assay) (BIO-RAD Laboratories, Milan, Italy) following the instructions provided by the manufacturer. Data concerning reactions were acquired by a Bio-Plex 200 reader, while a digital processor and Bio-Plex Manager® 6.0 software converted data into Median Fluorescence Intensity and Concentration (pg/ μ L) (BIO-RAD Laboratories, Milan, Italy) [19]. We considered only the cytokine values determined at the end of TBI treatment. TA values were eventually related to the inflammatory cytokine levels.

Follow-up

After discharge, patients were followed up monthly in the first six months and then every 6 to 12 months, in case of an uneventful post-transplant path. Follow-up duration was calculated from the date of HSCT to that of the patient's last visit or death. A minimum of 1-year follow-up for survivors was considered.

Endpoints

The primary endpoint was to find a correlation between the maximum TA value during TBI treatment and OS, as well as other transplant outcomes. The outcomes were reported at the early and the late phases after HSCT. The post-transplant time phases were previously defined [20]. Toxicity was graded according to National Cancer Institute common toxicity criteria [21]. The secondary endpoint was to determine a TA cut-off value that could better predict adverse transplant outcomes.

Statistics

Descriptive statistics were used to determine the distribution and frequency of the variables. Continuous variables were expressed as median and confidence interval (CI) between second and third quartiles (percentile 25 and percentile 75) or mean \pm standard deviation when appropriate, while categorical variables were expressed as frequency and absolute or a percentage value. Box and whisker plots were generated to display the numeric variables' distribution. Mann-Whitney test was performed to compare numeric variables between two different groups of patients. Kruskal-Wallis test was used for multiple comparisons between more than two groups. Fisher's test was adopted to analyse categorical variables in different groups of patients. We investigated the TA's validity in predicting transplant outcomes by assessing their respective Area Under the Curve (AUC) and Receiver Operating Characteristic (ROC) curve. Youden index was employed to establish the best cut-off for the sensitivity and specificity of each variable. Kaplan–Meier plots were generated for a graphical explanation of clinical outcomes, and Log-rank test was used to compare survival curves. *P*-values < 0.05 were considered as statistically significant. Statistical analyses were performed using WinStat (v.2012.1; In der Breite 30, 79189 Bad Krozingen, Germany) and MedCalc (Statistical Software version 18.9.1, Ostend, Belgium; <http://www.medcalc.org>; 2018).

Results

Patients

209 patients underwent an allogeneic HSCT from January 2000 to November 2019. 78 of them had an ALL and underwent a TBI-based conditioning before HSCT, thus becoming eligible for the study. The median follow-up was 5.63 years (range 1.08–19.83 years). Patients' and transplants' features are shown in Table 1.

OS after HSCT at last follow-up was 62.8% (49 patients). No demographic or transplant-related predictors such as gender, age at transplant, donor type, graft source, TBI protocol, or TBI-associated chemotherapy were related to OS.

57 patients (73.1%) received TBI with fractioned doses of 12 Gy (standard protocol), while 21 patients (26.9%) took a single TBI dose of 7.5 Gy. Mean dose-rate \pm standard deviation (SD) was 14.0 ± 2.0 cGy/min in the 7.5 Gy group and 18.7 ± 1.7 cGy/min in the 12 Gy group. The mean percentage variation \pm SD in dosimetry was $1.5 \pm 1.0\%$ and $-0.9 \pm 1.9\%$ in the two groups, respectively, under the acceptable 10% range [5].

Abnormal levels of TA during the TBI treatment were found in 71 patients (91%), and the highest values of TA were observed after the first day of TBI. The maximum TA value was 2210 U/L, documented before the third session. The mean TA value after the first day of TBI was $341 \text{ U/L} \pm 344 \text{ U/L}$, while the median value was 246 U/L.

Comparing the maximum TA values in the patients who underwent the two different TBI protocols, assessed before the third radiotherapy session, did not reveal statistically significant differences ($P = 0.2111$). Indeed, the mean TA value in the 7.5 cGy group was 305 U/L (range 46–2210 U/L), while in the 12 cGy group it was 223 U/L (range 25–1337 U/L).

Relationship between maximum TA values and OS

Maximum TA values predicted the post-transplant OS (AUC = 0.773; 95% CI = 0.66–0.86; $P < 0.001$). The maximum value of Youden index was 374 U/L, with a corresponding sensitivity of 58.6% and specificity of 96%. The relationship between OS and maximum TA values is displayed in the corresponding ROC curve (Fig. 1A).

Establishing an arbitrary cut-off of 374 U/L, OS was 78% versus 11% for patients below this value versus 11% for those above it ($P < 0.0001$) (Fig. 1B).

Relationship between maximum TA values and causes of death

We analyzed the causes of death in the study population. All-cause mortality was 37.2% (29 patients). In particular, 48.3% of deaths were attributable to TRM, while the remaining 51.7% to disease progression. Regarding the stage of the disease at transplant in the deceased group, 26 patients (89.6%) had a late stage disease, and only 3 patients (10.4%) had an early stage malignancy. As for the specific causes that contributed to TRM, 2 patients (14.3%) died of GVHD, 8 (57.1%) of infectious complications, and 4 (28.6%) of transplant-related organ toxicity.

The mean values of TA, documented after the first day of TBI, showed statistically significant differences ($P < 0.0001$) comparing the different causes of death (Fig. 2). We did not find any significant difference when comparing the mean TA values of the deceased patients with late stage disease and those of the subjects with early stage disease ($P > 0.05$).

Relationship between maximum TA values and early transplant-related complications

Patients with TA above 374 U/L had severe mucosal damage (grade III-IV) more commonly than the other group (83% versus 25%, $P < 0.0001$). No differences were detected in the incidence of pulmonary, renal, and neurological TBI-related toxicity, febrile neutropenia or sepsis, fungal and virus infections, veno-occlusive disease, and I-II grade hepatic toxicity. We observed the higher incidence of the III-IV grade hepatic toxicity in patients with TA above 374 U/L (18% and 56%, respectively; $P < 0.05$).

Relationship between maximum TA values and late transplant-related complications

We found no differences between the group with TA above 374 U/L and the group with TA below 374 U/L in the onset of long-term transplant-related complications, such as chronic GVHD, hypothyroidism, pancreatic insufficiency, cataract, and growth hormone deficiency.

We evaluated maximum TA values' diagnostic performance in predicting the death by leukemia relapse, obtaining the specific AUC - ROC curve (Fig. 3A). Maximum TA values showed high predictive performance in identifying disease progression-related deaths (AUC = 0.865; 95% CI = 0.77–0.93; $P < 0.0001$). The cut-off level of 374 U/L was both highly sensitive (80%) and specific (88.9%). The distribution of TA values is shown in Fig. 3B.

Relationship between maximum TA values and inflammation status

47 patients (81%) of the group with TA above 374 U/L and 15 patients (75%) of the other group underwent the cytokine assays at the end of TBI treatment. The majority of the various pro-inflammatory mediators analyzed had an abnormal concentration in both groups. We found no statistically significant differences for all the cytokines and chemokines evaluated between the two groups, except for tumor necrosis factor (TNF)- α , interleukin (IL)-6, and RANTES (regulated upon activation, normal T cell expressed, and secreted) ($P > 0.05$). In fact, in the second group, the concentrations of TNF- α , IL-6 and RANTES were significantly above the reference range and higher than those observed in the patients of the first group ($P < 0.0001$, $P < 0.0001$ and $P < 0.001$, respectively). The relative differences of IL-6, TNF- α , and RANTES post-TBI concentrations between the two groups are displayed in Fig. 4.

Discussion

The success of radiotherapy in eradicating tumors depends on the amount of total radiation dose provided, which is limited by the tolerance of normal tissues within the treatment volume, particularly those late-responding [22]. Different tissues take different times to express damage. As hematopoietic tissue, acute-responding tissues have high stem cell activity and high regenerative capacity [23]. In non-proliferative tissue, such as the liver or lung, the clinical expression of radiation injury can be delayed by months [24].

The occurrence of hyperamylasemia after parotid irradiation is a known phenomenon [25]. Salivary amylase secretion rapidly increases within a few hours after irradiation and reaches its peak within 12–36 hours [26]. Parotid glands belong to the tissue group with high radiosensitivity, despite being made by secretory cells with a slow turnover [27]. This peculiarity can be explained with the massive release of secretory granules rich in proteolytic enzymes during radiation-related destruction of serous cells [26]. This study identified abnormal TA levels in 91% of patients after 24 hours of TBI onset. The patients

consistently showed rapid TA increase following irradiation but with extreme individual variability, and only 12% of them have incredibly high amylasemia (> 500 U/L). Our study does not demonstrate any correlations between the different TBI protocols and the degree of parotid response to irradiation damage.

Human response to radiation is widely different due to individual cellular radiosensitivity, primarily determined by genetic factors [28, 29]. However, the molecular basis of individual radiosensitivity remains poorly understood [30].

We investigated whether TA matches the characteristics of a reliable marker of individual radiosensitivity. Analyzing the early and long-term treatment-related complications, we did not find the relationship between the rises of TA values and clinically express irradiation damage, neither acute nor late-responding tissues, except severe mucosal damage digestive system and III-IV grade acute liver injury. The relationship between mucosal and parotid irradiation damage is intuitive because both are early-responding tissues. Acute liver injury is most likely attributable to chemotherapy medications, which are part of the myeloablative pre-transplant conditioning.

Remarkably, these data show a strong relationship between the cumulative incidence of death and irradiation-related TA values. Surprisingly, disease progression-related mortality was the most common cause of death in patients with TA above 374 U/L. A less significant relapse rate would have been the most likely result, considering the high damage suffered by the bone marrow and, consequently, by leukemic stem cell (LSC) microenvironment.

Various mechanisms might be involved in LSC's irradiation resistance. According to most of the data reported in the literature, leukemic cells are radiosensitive with a D_0 values between 0.8 and 1.5 Gy, like bone marrow cells. However, some studies report a wide range of leukemia cells' radiosensitivity with D_0 values varying from 0.3 Gy to 4 Gy [31]. Leukemia cells response ranges from remarkable radiosensitivity to considerable intrinsic radioresistance. If leukemia cells in vivo vary to the same degree as they do in vitro, a TBI regimen of 7×2 Gy will produce a spectrum of surviving fractions ranging from 10^{-2} to 10^{-21} with a median of about 10^{-5} . It is therefore possible that radiation damage might select some specific, aggressive leukemic clones, making the malignancy harder to treat [32].

Another mechanism that has role in explaining the TBI failure to eradicate leukemia is the capacity of some LSC populations to repair sublethal radiation damage [33]. Several studies demonstrated an increase in the survival of leukemic cells with fractionated irradiation schedules [34].

If we relate high TA values with higher radiosensitivity and higher disease progression related to mortality, the possible causes are likely to be connected to the LSC microenvironment.

One of the bone marrow (BM) microenvironment elements are specialized stromal niches, where hematopoietic stem cells (HSCs) are allocated. The function of these niches is to support HSC self-renewal and multipotency [35]. TBI severely damages the BM stroma with its hematopoietic niches.

Trabecular bone volume loss and microstructure damage are present as early as one week after irradiation [36]. The 90% of irradiated clonogenic BM stroma progenitor cells are permanently lost or lose the multi-lineage differentiation capacity [37]. Loss of stroma function prevents successful HSC engraftment and delays the recovery of innate and adaptive immunity. The main aim of allogeneic HSCT is to activate the donor's alloreactive immune cells against the patient's leukemia, the immune process known as the graft-versus-leukemia effect [38]. In case of incomplete or delayed reconstruction of immunocompetent donor cells, the transplant's main function is missing, and primary disease relapse occurs.

A further important consideration is the systemic inflammatory response that affects most of the tissues due to whole-body radiation [39]. Our data shows that the concentration of proinflammatory cytokines as TNF- α , IL-6, and RANTES is significantly higher in the group with TA values > 374 U/L. Inflammatory cytokines such as IL-1, IL-6, IL-17, and TNF- α are known to be highly elevated within 24 to 48 hours of radiation exposure [36, 40]. The role of these cytokines in bone resorption is widely described [41]. Increased RANTES expression is associated with a wide range of inflammatory disorders [42]. During resorption, the bone delivers numerous growth factors stored in the bone matrix. The released cytokines render the bone microenvironment particularly favourable to cancer cell survival [43, 44].

This study has some limits. First of all, this is a retrospective consecutive case series of subjects collected from a large time interval, which is almost 20 years long, and the number of patients with very high TA is overall limited. However, it must be specified that TBI, with the exception of the switch from single high-dose administrations to fractionated regimens, have substantially remained the same in the last 20 years. TBI's progression has been more about the quality of the technologies and thus of the machines that provide radiations, rather than the draft of new delivery protocols. Moreover, the largest cohort was needed to achieve a reasonable degree of statistical significance.

Remarkably, this is the first study evaluating irradiation-induced TA values' performance in identifying highly radiosensitive individuals and predicting early and long-term transplant-related outcomes.

Conclusion

Despite the well-known individual heterogeneity in radiation susceptibility, TBI protocols have not yet considered it [45]. Precision medicine is ambitiously trying to identify biomarkers and mediators that might be able to predict the interindividual sensitivity to radiation. These elements would be extremely useful not only in the first, delicate phases of the HSCT procedure, when radiation toxicity can put a patient's life at serious risk, but also on a long term, since pediatric cancer survivors have now a longer life expectancy and might be affected by radiation consequences even decades after TBI. TA might play this role, and serve as a low-cost indicator of a genetic predisposition to radiation toxicity, identifying those who are at greater risk of developing radiation consequences.

Abbreviations

HSCT - hematopoietic stem cell transplantation; TBI – total body irradiation; ALL - acute lymphoblastic leukemia; TA – total amylase; OS – overall survival; GVHD - graft-versus-host disease; TRM - transplant-related mortality; TLD - thermoluminescence dosimetry; MOSFET - metal oxide semiconductor field effect transistor; U/L - unity per liter; CI - confidence interval; AUC - area under the curve; ROC - Receiver Operating Characteristic; Sd - standard deviation; TNF - tumor necrosis factor; IL – interleukin; RANTES - regulated upon activation normal T cell expressed and secreted; LSC - leukemic stem cell; MB - bone marrow; HSCs - hematopoietic stem cells.

Declarations

Ethics approval and consent to participate:

The clinical study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the institute (reference IRB-BURLO no. 03/2020) approved the study protocol. The parents of all subjects enrolled signed written consent for the collection and use of their personal data.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

FB wrote the manuscript. RS carried out the statistical design and analysis. AM performed in vitro assays. RV and FC carried out in vivo procedures. DZ and AM discussed results and created survival curves. EB reviewed the manuscript. NM designed present study and did the follow-up of patients.

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Table

Table 1 not available with this version.

Figures

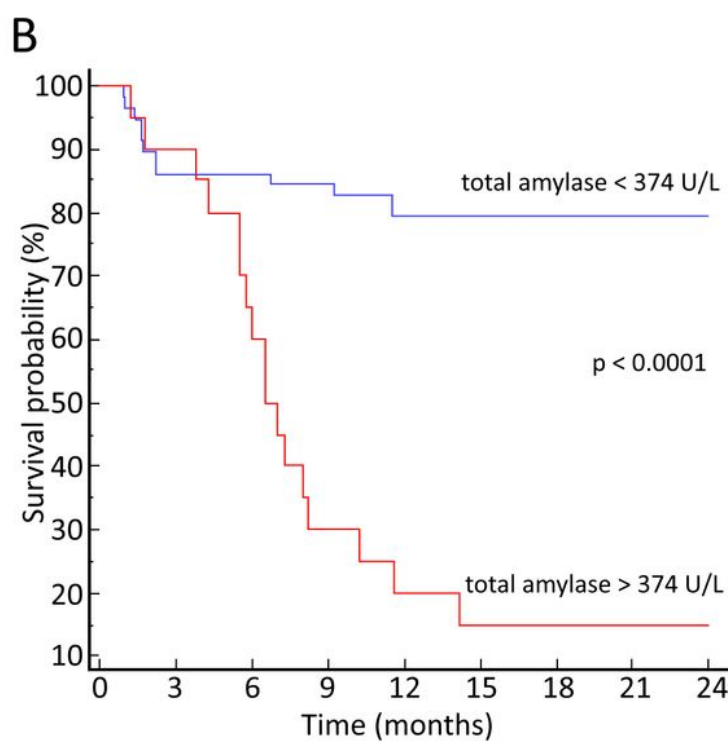
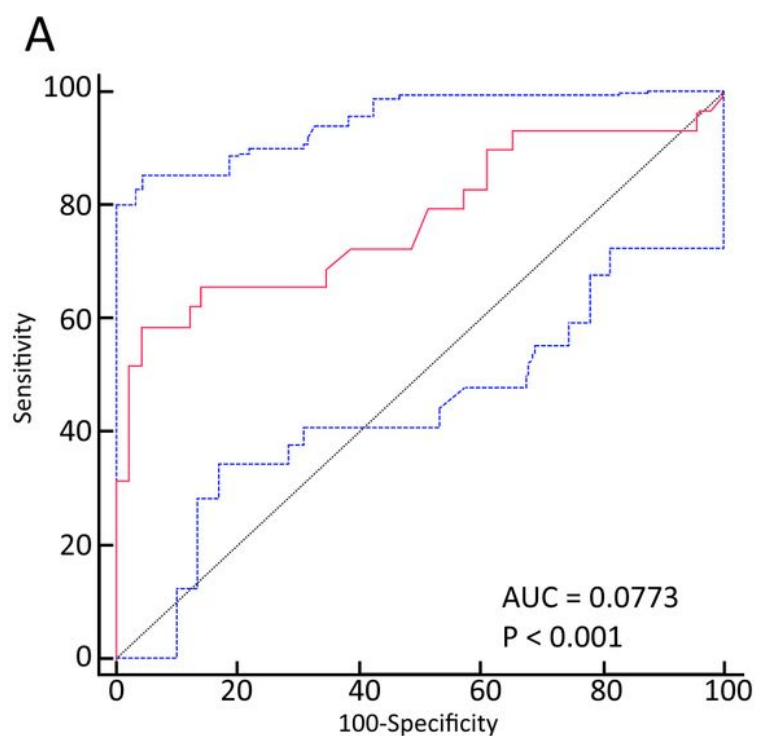


Figure 1

(A) Receiver operative characteristics curves of total amylase diagnostic performance in predicting the overall survival after hematopoietic stem cell transplantation with total body irradiation-based conditioning. (B) Kaplan-Meier curves for overall survival of patients with total amylase values below 374 U/L (blue line) and above 374 U/L (red line).

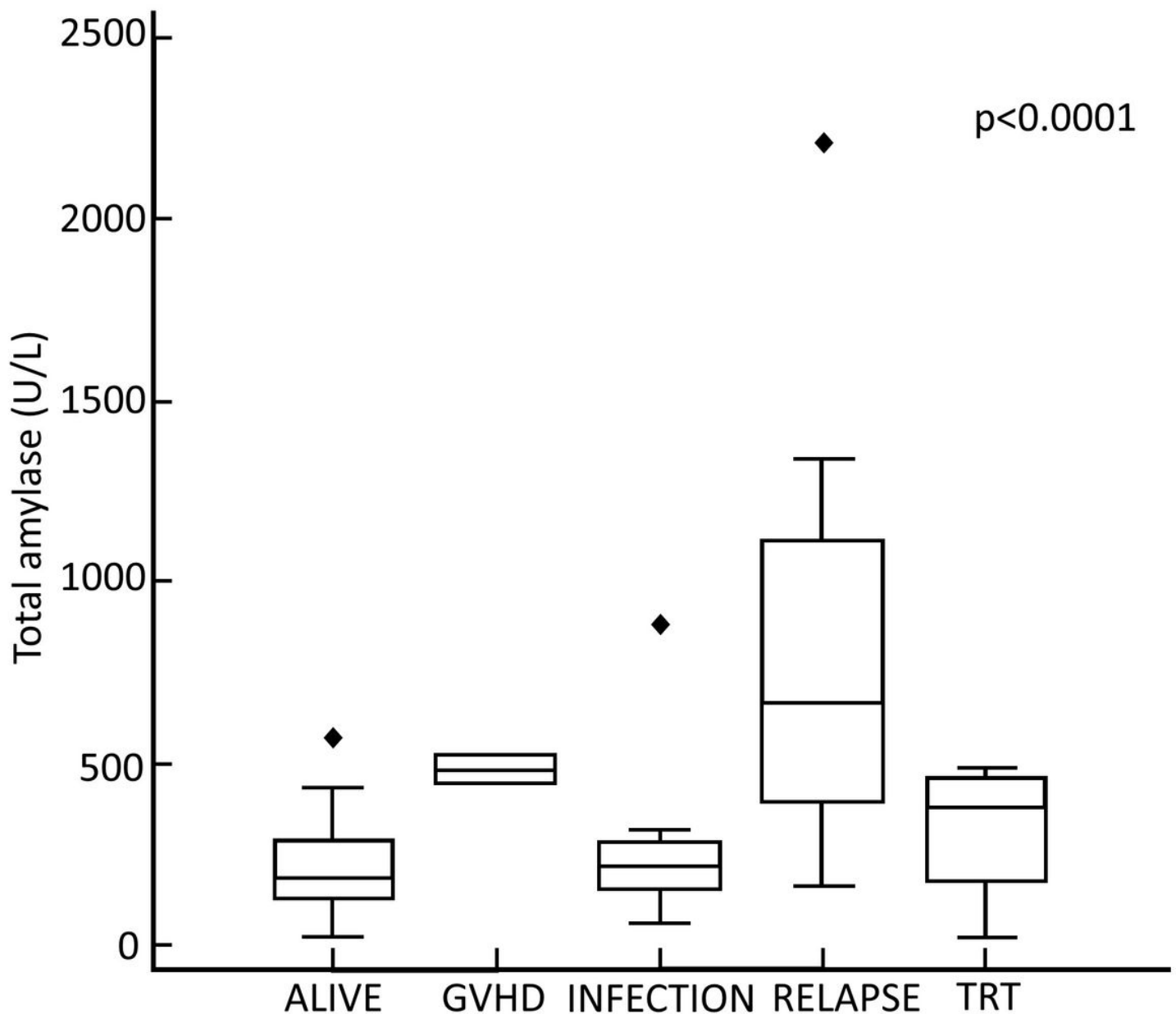


Figure 2

Box and Whisker Plot of maximum total amylase concentration in patients with different transplant-related outcomes. Box plots showing the median (line), upper and lower quartiles (box), and 5% and 95% limits (lines extending from the box). The outcomes are shown on the x-axis. From left to right of the axis: surviving patients, dead of graft versus host disease (GVHD), dead of infection, dead of leukemia relapse, dead of transplant-related toxicity (TRT). The highest TA value was observed in the group of patients who died from a leukemic relapse (673 U/L), followed by death due to GVHD (490 U/L) and by transplant-

related organ toxicity (386 U/L). The lowest TA value was detected in the group of patients who died from infectious complications (215 U/L). A Kruskal-Wallis test confirmed the significant difference in TA values of confronted groups ($p < 0.000061$).

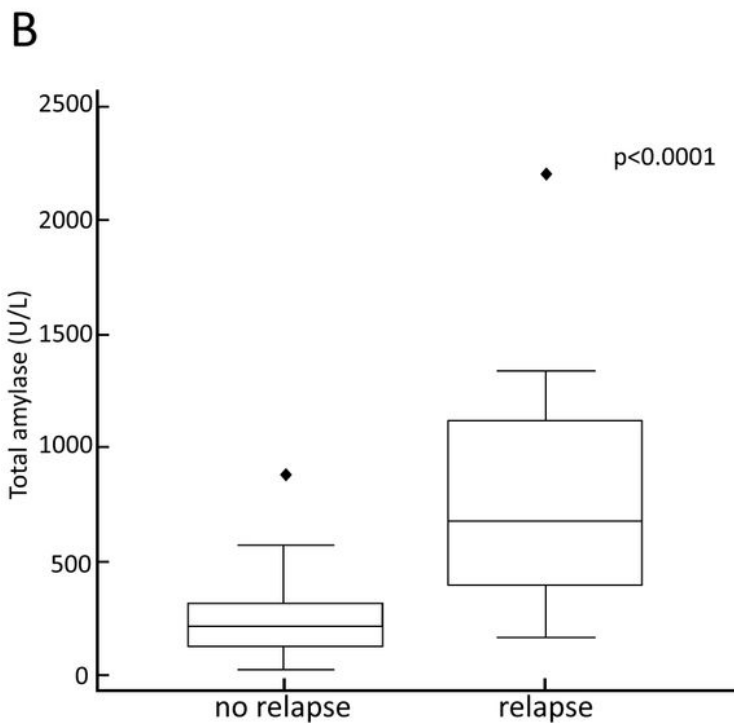
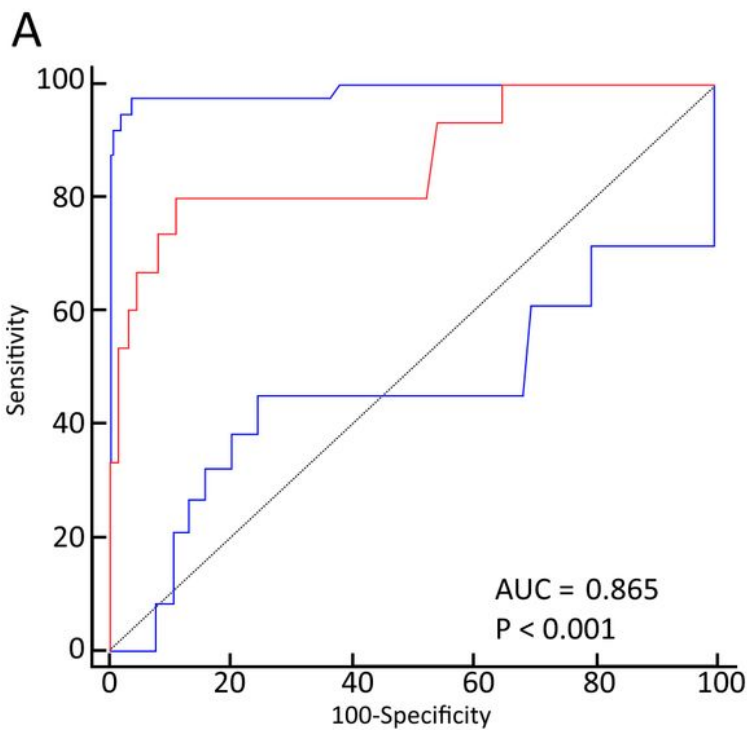


Figure 3

(A) Receiver operative characteristics curves of total amylase diagnostic performance in predicting leukemia relapse-related death after hematopoietic stem cell transplantation with total body irradiation-

based conditioning. (B) Box and Whisker Plot of maximum total amylase values of recurrence-free patients and patients with disease recurrence.

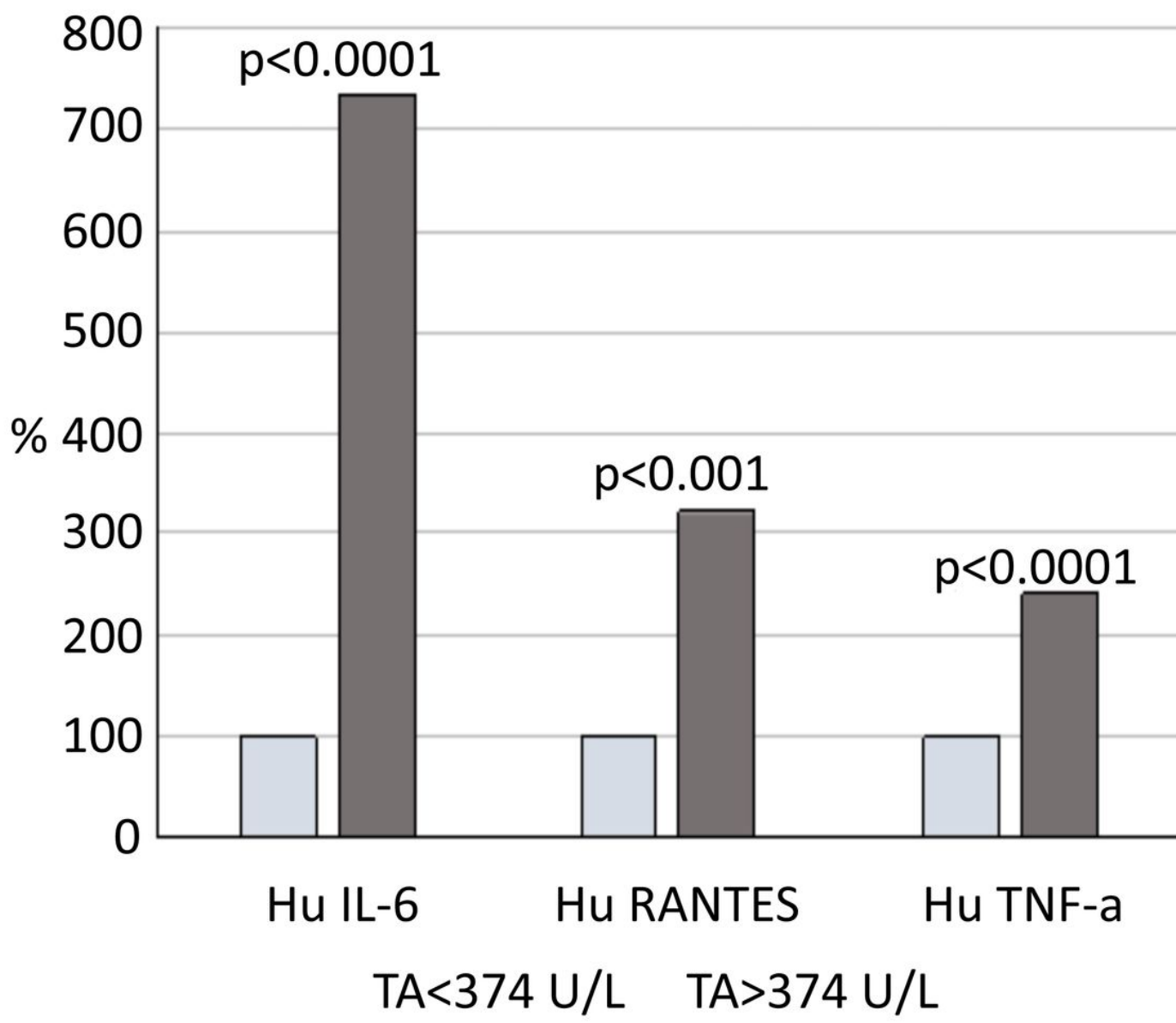


Figure 4

Graphical presentation of relative differences in proinflammatory cytokines values after total body irradiation between the patients with total amylase values below and above 374 U/L. Abbreviations: Hu IL-6 – human interleukin 6, Hu RANTES – human regulated upon activation normal T cell expressed and secreted cytokine, Hu TNF-α – human tumor necrosis factor α, TA – total amylase.