UČESTALOST SLABOSTI KALEMA NAKON ALOGENE TRANSPLANTACIJE MATIČNIH ĆELIJA HEMATOPOEZE

ORIGINALNI RAD

ORIGINAL ARTICLE

THE FREQUENCY OF POOR ENGRAFTMENT AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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SAŽETAK

Uvod: Slabost kalema predstavlja jednu od mogućih komplikacija nakon alogene transplantacije matičnih ćelija hematopoeze (alo-TMĆH). Manifestuje se kao pancitopenija ili bicitopenija, sa potpunim ili nepotpunim donorskim himerizmom. Tri entiteta slabosti kalema su: slaba funkcija kalema (engl. *poor graft function – PGF*), slabost kalema u užem smislu (engl. *graft failure – GF*) i odbacivanje kalema (engl. *– graft rejection – GR*).

Cilj: Cilj ovog istraživanja je da pokaže učestalost slabosti kalema kod pacijenata, nakon alogene transplantacije matičnih ćelija hematopoeze, u periodu od 20. decembra 2017. godine do 25. decembra 2020. godine, na Klinici za hematologiju Univerzitetskog kliničkog centra Srbije (UKCS), kao i učestalost svake vrste slabosti pojedinačno, radi boljeg rešavanja i razumevanja ove ozbiljne komplikacije.

Materijali i metode: U retrospektivnu kohortnu studiju je uključeno 58 bolesnika. Slabost kalema je dijagnostikovana kao pancitopenija (granične vrednosti: hemoglobin < 70g/l; *ANC* < 0,5 x 10^9/L; broj trombocita < 20 x 10^9/L), tri dana zaredom, od D+28, pri isključenju teškog oblika *GvHD*-a i relapsa, sa potpunim donorskim himerizmom kod *PGF*-a, a sa nepotpunim donorskim himerizmom kod *GF*-a. *GR* je dijagnostikovan prilikom akutnog odbacivanja kalema od strane recipijenta sa aplazijom koštane srži ili pancitopenijom.

Rezultati: Slabost kalema je imalo 13 (22,4%) pacijenata. Najveću zastupljenost je imao *PGF* sa sedam slučajeva (12,1% od 58 pacijenata; 53,8% od 13 pacijenata), dok je *GF* bio zastupljen sa tri slučaja (5,2% od 58 pacijenata; 23,1% od 13 pacijenata), kao i *GR*, koji se takođe desio u tri slučaja (5,2% od 58 pacijenata; 23,1 % od 13 pacijenata). Preživljavanje pacijenata sa slabošću kalema je bilo pet meseci, dok je za pacijente bez slabosti kalema bilo 57 meseci.

Zaključak: Sva tri tipa slabosti moraju se jasno diferencijalno dijagnostikovati u odnosu na himerizam, jer definisanje tačnog tipa slabosti može značajno da utiče na terapijski pristup pacijentu i konačni ishod.

Ključne reči: slaba funkcija kalema, slabost kalema. odbacivanje kalema, alo-TMĆH

ABSTRACT

Introduction: Poor engraftment represents one of the possible complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It presents as pancytopenia or bicytopenia, with or without complete donor chimerism. There are three entities of poor engraftment: poor graft function (PGF), graft failure (GF), and graft rejection (GR).

Aim: This study aims to show the frequency of poor engraftment, as well as the frequency of all of its entities individually, among the patients of the Clinic of Hematology of the University Clinical Center of Serbia (UCCS), who underwent allo-HSCT between December 20, 2017 and December 25, 2020, for the purpose of achieving improved management and understanding of this serious complication.

Materials and methods: This retrospective cohort study included 58 patients. Diagnosis of poor engraftment was confirmed by pancytopenia (cut off values: hemoglobin < 70g/L; platelet count < 20 x 10^9/L; absolute neutrophil count (*ANC*) < 0.5 x 10^9/L), for three consecutive days, as of day D+28, with the exclusion of severe graft versus host disease (GvHD) and relapse, with complete donor chimerism in PGF and with incomplete donor chimerism in GF. GR presented as acute rejection of the graft by the recipient with bone marrow aplasia or pancytopenia.

Results: Poor engraftment was confirmed in 13 of 58 patients (22.4%). Patients with *PGF* were the majority, with 12.1% (seven patients), while patients with *GF* and those with *GR* had the same incidence of 5.2% (three patients). Overall survival for patients with poor engraftment after allo-HSCT was five months, which is significantly less than the overall survival of the patients who had good engraftment after allo-HSCT (57 months).

Conclusion: The three types of poor engraftment must be precisely discriminated and diagnosed in relation to donor chimerism in order to decrease morbidity and mortality in patients, post allo-HSCT.

Keywords: poor graft function, graft failure, graft rejection, allo-HSCT

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UVOD

Alogena transplantacija matičnih ćelija hematopoeze (alo-TMĆH) je zamena i repopulacija hematopoeznog tkiva primaoca hematopoeznim tkivom davaoca, odnosno druge osobe, koja može biti u srodstvu ili se pronalazi pretragom registra donora. Predstavlja metodu lečenja visokorizičnih hematoloških oboljenja kao što su: akutna mijeloidna leukemija (AML), akutna limfoblastna leukemija (ALL), mijelodisplastični/mijeloproliferativni sindrom (MDS/MPN), teška aplastična anemija (engl. *severe aplastic anemia – SAA*), Hočkinov limfom (HL) i Nehočkinov limform (NHL).

Nakon uspešne alogene transplantacije, pored relapsa, može doći i do pojave komplikacija, kao što su bolest kalema protiv domaćina (engl. *graft versus host disease – GvHD*), te razne forme slabosti kalema.

Slabost kalema se manifestuje kao pancitopenija sa potpunim donorskim himerizmom (slaba funkcija kalema – *poor graft function* (*PGF*)), pancitopenija sa nepotpunim donorskim himerizmom (slabost kalema u užem smislu – *graft failure* (*GF*)), ili se javlja odbacivanje transplantata (*graft rejection* (*GR*)), što se manifestuje aplazijom koštane srži i/ili pancitopenijom [1–3].

PGF je životno ugrožavajuća komplikacija, koja se karakteriše gubljenjem ćelija dve ili tri ćelijske krvne linije sa hipoplazijom koštane srži, pri čemu postoji potpuni donorski himerizam. Sa naglim razvojem alogene TMĆH, posebno haploidentične-TMĆH, *PGF* je postao aktuelan problem. Najčešći faktori rizika za razvoj *PGF*-a su: niska doza donorskih CD34+ ćelija, donor specifična antitela (DSA), citomegalovirusna (CMV) infekcija, bolest kalema protiv domaćina (engl. *graft versus host disease – GvHD*), prevelika zasićenost gvožđem, splenomegalija, i drugi faktori [1–7].

GF se definiše kao nedostatak donorskih ćelija posle engraftmenta, prilikom alogene TMĆH. Može biti primarni i sekundarni.

Primarni *GF* se definiše kao nepotpuni engraftment i oporavak donorskih ćelija kod pacijenta, unutar prvog meseca posle alogene TMĆH, bez prisutnog relapsa bolesti. Sekundarni *GF* se odnosi na gubitak prethodno funkcionalnog grafta, koji rezultuje citopenijom, u barem dve ćelijske linije. Primarni *GF* je češće povezan sa težom kliničkom slikom i lošijom prognozom nego sekundarni [8].

GR se, kao termin, odnosi na imunski posredovanu reakciju zaostalih ćelija domaćina na donorske ćelije, te je stoga povezan samo sa alogenom TMĆH. Često se u stranoj literaturi navodi zajedno sa *GF*-om, kao njegov podentitet [8,9].

Pregledom raspoložive literature, može se videti da su slabost kalema i njena učestalost jedne od vodećih tema u poslednjih desetak godina, kada je u pitanju

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the replacement and repopulation of the hematopoietic tissue of the recipient with the hematopoietic tissue of another person, who may be related to the recipient or is found through a search in the donor registry. It is a method of treating high-risk hematological diseases, such as the following: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic/myeloproliferative syndrome (MDS/MPN), severe aplastic anemia (SAA), Hodgkin's lymphoma (HL), and Non-Hodgkin's lymphoma (NHL).

Upon successful allogeneic transplantation, in addition to relapse, complications, such as graft versus host disease (GvHD) or different forms of poor engraftment, may occur.

Poor engraftment manifests as pancytopenia with complete donor chimerism (poor graft function (PGF)), pancytopenia with incomplete donor chimerism (graft failure (GF)), or graft rejection (GR) can occur, which manifests as bone marrow aplasia and/or pancytopenia [1–3].

PGF is a life-threatening complication, characterized by cell loss in two or three blood cell lineages with bone marrow hypoplasia and the occurrence of complete donor chimerism. With the rapid development of allogeneic HSCT, especially haploidentical-HSCT, PGF has become an important ongoing problem. The most common risk factors for the development of PGF are as follows: low dose of donor CD34+ cells, donor specific antibodies (DSA), cytomegalovirus (CMV) infection, graft versus host disease (GvHD), excess iron saturation, splenomegaly, and other factors [1–7].

GF is defined as the lack of donor cells upon engraftment in allo-HSCT. It may be primary or secondary.

Primary GF is defined as incomplete engraftment and recovery of donor cells in the patient within the first month following allo-HSCT, without the occurrence of relapse. Secondary GF relates to the loss of a previously functional graft, resulting in cytopenia, in at least two cell lineages. Primary GF is more frequently linked to severe clinical presentation and a poorer prognosis than secondary GF [8].

As a medical term, GR relates to the immune-mediated reaction of residual host cells to donor cells, which is why it is linked only to allo-HSCT. In international literature, it is often noted along with GF, as its subentity [8,9].

Review of available literature shows that poor engraftment and its incidence have been one of the leading topics regarding allogeneic transplantation, in the previous ten years. A study by Zhao et al., carried out in the period between 2014 and 2018, shows that the presence of PGF was confirmed in 43 out of 830 patients who had undergone allo-HSCT [1]. The incidence

alogena transplantacija. Studija koju su sproveli Žao i saradnici u periodu od 2014. do 2018. godine, prikazuje da je prisustvo *PGF*-a potvrđeno kod 43 pacijenta od 830 bolesnika koji su imali alogenu TMĆH [1]. Incidencija *GF*-a varira u zavisnosti od modaliteta transplantacije, te različitih studija i izveštaja [8]. Analizom 23.272 pacijenta iz baze Centra za međunarodno istraživanje presađivanja krvi i koštane srži (engl. *Center for International Blood and Marrow Transplant Research – CIBMTR*) zabeležena je incidencija *GF*-a od 5,5 %, 2015. godine, dok je godinu dana kasnije, u velikoj retrospektivnoj kohortnoj studiji, koja je obuhvatila 4.684 nesrodne alogene transplantacije (2006 - 2012) potvrđena incidencija *GF*-a od 3,8% [9].

Sagledavajući ovakve rezultate drugih studija, cilj ovog rada je da pokaže učestalost slabosti kalema kod pacijenata koji su imali alogenu TMĆH u periodu od 20. decembra 2017. do 25. decembra 2020. godine, na Klinici za Hematologiju Univerzitetskog kliničkog centra Srbije (UKCS), kao i učestalost svake vrste slabosti pojedinačno, u cilju razumevanja ovog aktuelnog problema i poboljšanja njegovog rešavanja.

MATERIJALI I METODE

U retrospektivnu kohortnu studiju je uključeno 58 bolesnika sa Klinike za Hematologiju UKCS-a, od toga 33 muškog i 25 ženskog pola, sa medijanom starosti 38 godina, u opsegu od 19 do 59 godina. Od 58 pacijenata, njih petoro je umrlo pre engraftmenta. Bolesnici su imali sledeće dijagnoze: Hočkinov limfom (HL), Nehočkinov limfom (NHL), akutna limfoblastna leukemija (ALL), akutna mijeloidna leukemija (AML) i mijelodisplastični/mijeloproliferativni sindrom (MDS/MPN).

Prema protokolu, za dan engraftmenta uziman je dan u kome je broj neutrofila bio iznad 1 x 10^9 i broj trombocita iznad 20 x 10^9, u tri uzastopna dana, bez prethodne transfuzije trombocita. Kondicioni režimi predstavljaju primenu hemioterapijskih agenasa i/ili zračne terapije pre alogene TMĆH, a po svojoj jačini se mogu podeliti na mijeloablativne kondicione režime (engl. *myeloablative conditioning – MAC*), koji dovode do potpunog opustošenja koštane srži, i kondicione režime redukovanog intenziteta (engl. *reduced-intensity conditioning*, *RIC*), koji koriste manje doze zračenja i hemioterapijskih agenasa.

Za profilaksu infekcija u alogenoj TMĆH korišćen je sledeći protokol:

1. Antibakterijska profilaksa (hinolon levofloksacin, 500 mg *per os* (p.o.))
2. Trimetoprim–sulfametoksazol, 2 x 400 mg p.o., do D+90 (protiv: *Toxoplasma gondii* i *Pneumocystis jirovecii*)
3. Antivirusna profilaksa: aciklovir, 3 x 400 mg p.o.
4. Antifungalna profilaksa: mikafungin, 50 mg i.v., dve nedelje, od D0 do engraftmenta, a potom posakonazol, 3 x 200 mg p.o. (sol.) do D+90

of GF varies depending on the transplantation modality, as well as on the different studies and reports [8]. Analysis of 23,272 patients from the Center for International Blood and Marrow Transplant Research (CIBMTR) database demonstrated a GF incidence of 5.5 %, in 2015, while a year later, in a large retrospective cohort study, which included 4,684 unrelated allogeneic transplantations (2006 - 2012), an GF incidence of 3.8% was confirmed [9].

Bearing in mind the results from previous studies, the aim of this paper is to demonstrate the incidence of poor engraftment in patients who underwent allo-HSCT in the period between December 20, 2017 and December 25, 2020, at the Clinic of Hematology of the University Clinical Center of Serbia (UCCS), as well as to demonstrate the incidence of each type of poor engraftment individually, for the purpose of understanding this ongoing problem more thoroughly and improving the way that it is resolved.

MATERIALS AND METHODS

This retrospective cohort study involved 58 patients treated at the Clinic of Hematology of the UCCS, of whom 33 male and 25 female subjects, of a median age of 38 years (range: 19 – 59 years). Of the 58 patients, five died before engraftment. The patients were diagnosed with the following diseases: Hodgkin's lymphoma (HL), Non-Hodgkin's lymphoma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and myelodysplastic/myeloproliferative syndrome (MDS/MPN).

According to protocol, the day when the neutrophil count was 1 x 10^9 and the platelet count was above 20 x 10^9, for the third consecutive day, without previous thrombocyte transfusion, was taken as the day of engraftment. The conditioning regimens represent the application of chemotherapeutic agents and/or radiation therapy prior to allogeneic HSCT, and according to their intensity they can be categorized as myeloablative conditioning (MAC) regimens, which lead to complete bone marrow ablation, and reduced-intensity conditioning (RIC) regimens, which use lower doses of radiation and chemotherapeutic agents.

For infection prophylaxis in allo-HSCT, the following protocol was observed:

1. Antibacterial prophylaxis (the quinolone levofloxacin, 500 mg *per os* (p.o.))
2. Trimethoprim-sulfamethoxazole, 2 x 400 mg p.o., until D+90 (against: *Toxoplasma gondii* and *Pneumocystis jirovecii*)
3. Antiviral prophylaxis: acyclovir, 3 x 400 mg p.o.
4. Antifungal prophylaxis: micafungin, 50 mg IV, for two weeks, as of D0 until engraftment, then posaconazole, 3 x 200 mg p.o. (sol.) until D+90

Prema važećem protokolu, prevencija *GvHD*-a se sprovodila primenom imunosupresivne terapije (IST), u sklopu kondicioniranja antitimocitnim globulinom (ATG), (doza za nesrodnu transplantaciju: 20 mg/kg TM; doza za srodnu transplantaciju: 10 mg/kg TM, D–3 do D–1), uz ciklosporin A, u dozi od 3 mg/kg TM, od D-1 do D+180, uz primenu *Seattle* protokola za srodne alogene transplantacije, i to: metotreksat, u dozi od 15 mg/m^2, D+1, i u dozi od 10 mg/m^2, D+3 i D+6, dok se kod nesrodne transplantacije davao i na D+11, u dozi od 10 mg/m^2.

U skladu sa važećim protokolom, u slučaju postojanja *GvHD*-a, nastavljana je profilaksa gljivičnih infekcija posakonazolom uz IST: ciklosporin A (CSA), koji je davan u dozi 3 mg/kg TM do D+180, a nakon toga se doza postepeno redukovala. Takođe, prema protokolu, IST lek može naknadno, u slučaju toksičnosti ciklosporina A, biti i takrolimus sa produženim dejstvom.

Prema važećim standardima, za dijagnozu slabosti kalema kod pacijenata, utvrđeno je postojanje pancitopenije (hemoglobin manji od 70 g/l; apsolutni broj neutrofila (engl. *absolute neutrophil count – ANC*) manji od 0,5 x 10^9/L; broj trombocita manji od 20 x 10^9/L), tri dana zaredom, od D+28, pri isključenju teškog GvHD-a i relapsa, sa potpunim donorskim himerizmom kod *PGF*-a, a sa nepotpunim kod *GF*-a. *GR* je dijagnostikovan prilikom akutnog odbacivanja kalema od strane primaoca sa aplazijom koštane srži ili pancitopenijom.

STATISTIČKA ANALIZA

Inicijalno je formirana baza podataka grupisanjem i tabeliranjem rezultata po ispitivanim obeležjima bolesnika. Deskriptivni statistički parametri izraženi su kroz medijanu, modus i raspodele relativnih frekvencija. Ukupno preživljavanje bolesnika obuhvatalo je period od momenta dijagnoze do smrtnog ishoda, odnosno zaključno sa 25. decembrom 2020. godine, kod živih bolesnika. Preživljavanje bolesnika, u odnosu na to da li su imali slabost kalema ili ne, analizirano je Kaplan-Majerovom metodom i upoređivano *log-rank* metodom. Za statističku obradu podataka, korišćen je softver *SPSS 23.0* za *Microsoft Windows*.

REZULTATI

U studiji od 58 pacijenata, 33 (56,9%) je bilo muškog a 25 (43,1%) ženskog pola, sa medijanom starosti od 38 godina (opseg: 19 – 59), (Prilozi I i II).

Tabela 1. Godine starosti bolesnika lečenih alogenom transplantacijom matičnih ćelija hematopoeze (alo-TMĆH)

Table 1. Age of patients treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT)

Medijana starosti / *Median age*	38
Najmlađi pacijent / *Youngest patient*	19
Najstariji pacijent / *Oldest patient*	58

In keeping with the standard protocol, prevention of GvHD was carried out through the implementation of immunosuppressive therapy (IST), within the conditioning regimen with antithymocyte globulin (ATG), (dose for unrelated transplantation: 20 mg/kg BM; dose for unrelated transplantation: 10 mg/kg BM, D–3 to D–1), with cyclosporine, at a dose of 3 mg/kg BM, from D-1 to D+180, with the application of the Seattle protocol for related allogeneic transplantations, namely: methotrexate, at a dose of 15 mg/m^2, D+1, and at a dose of 10 mg/m^2, D+3 and D+6, while in unrelated transplantation, it was administered on D+11, at a dose of 10 mg/m^2.

In keeping with the standard protocol, in case of the development of GvHD, antifungal prophylaxis was continued with posaconazole, along with IST: cyclosporine A (CSA), which was administered at a dose of 3 mg/kg BM until D+180, upon which the dose was gradually reduced. Also, according to protocol, IST medication can subsequently, in case of cyclosporin A toxicity, be replaced with prolonged-release tacrolimus.

According to the current standards, for the purpose of diagnosing poor engraftment in patients, the existence of pancytopenia was determined (hemoglobin level below 70 g/l; absolute neutrophil count (ANC) below 0.5 x 10^9/L; platelet count below 20 x 10^9/L), for three consecutive days, from D+28, with the exclusion of GvHD and relapse, with complete donor chimerism in PGF and incomplete donor chimerism in GF. GR was diagnosed in acute graft rejection by the recipient, with bone marrow aplasia and pancytopenia.

STATISTICAL ANALYSIS

Initially, a database was formed by grouping and tabulating results according to the patient characteristics being investigated. Descriptive statistical parameters were expressed as the median, mode, and relative frequency distribution. The overall patient survival covered the period from the moment of diagnosis until the lethal outcome, or until December 25, 2020, in living patients. Patient survival, in relation to whether the patients had poor engraftment or not, was analyzed with the Kaplan-Meier method and compared with the use of the log-rank method. The SPSS 23.0 software for Microsoft Windows was used for statistical data processing.

RESULTS

This study involved 58 patients, of whom 33 (56.9%) male and 25 (43.1%) female subjects, of a median age of 38 years (range: 19 – 59 years), (Appendices I and II).

Most patients had AML, a total of 26 (44.8%); followed by 18 (31%) ALL patients; next, there were eight HL (13.8%) patients; followed by three (5.1%) NHL patients; as well as also three (5.1%) MDS/MPN patients (Appendix III).

Tabela 2. Distribucija bolesnika lečenih alogenom transplantacijom matičnih ćelija hematopoeze (alo-TMĆH), prema polu

Table 2. Distribution of patients treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT), by sex

	n	Procenat / *Percentage* %
Muški / *Male*	33	56.9
Ženski / *Female*	25	43.1
Ukupno / *Total*	58	100.0

Najviše je bilo pacijenata sa AML-om, njih 26 (44,8%); zatim sa ALL-om, njih 18 (31%); potom sa HL-om, osam (13,8%) pacijenata; sa NHL-om, njih troje (5,1%), te sa MDS/MPN-om, takođe troje (5,1%) pacijenata (Prilog III).

Nakon alogene TMĆH, očekivano preživljavanje je iznosilo 57 meseci (Prilog IV). Na D+30, potpuni donorski himerziam je postiglo 45 pacijenata (77,6%). Kondicioni režimi bili su *RIC* i *MAC* i to najviše *MAC* u odnosu na *RIC* – *MAC*: 74,1% (43 od 58 pacijenata); *RIC*: 25,9% (15 od 58 pacijenata), (Prilog V).

Slabost kalema je imalo 13 (22,5%) pacijenata od ukupnog broja obuhvaćenog studijom, a zastupljenost *PGF*-a je iznosila sedam pacijenata (12,1% od 58 pacijenata; 53,8% od 13 pacijenata). Zastupljenost *GF*-a je bila tri pacijenta (5,2% od 58 pacijenata; 23,1% od 13 pacijenata), a zastupljenost *GR*-a je takođe iznosila tri pacijenta (5,2% od 58 pacijenata; 23,1% od 13 pacijenata), (Prilog VI). Očekivano preživljavanje paci-

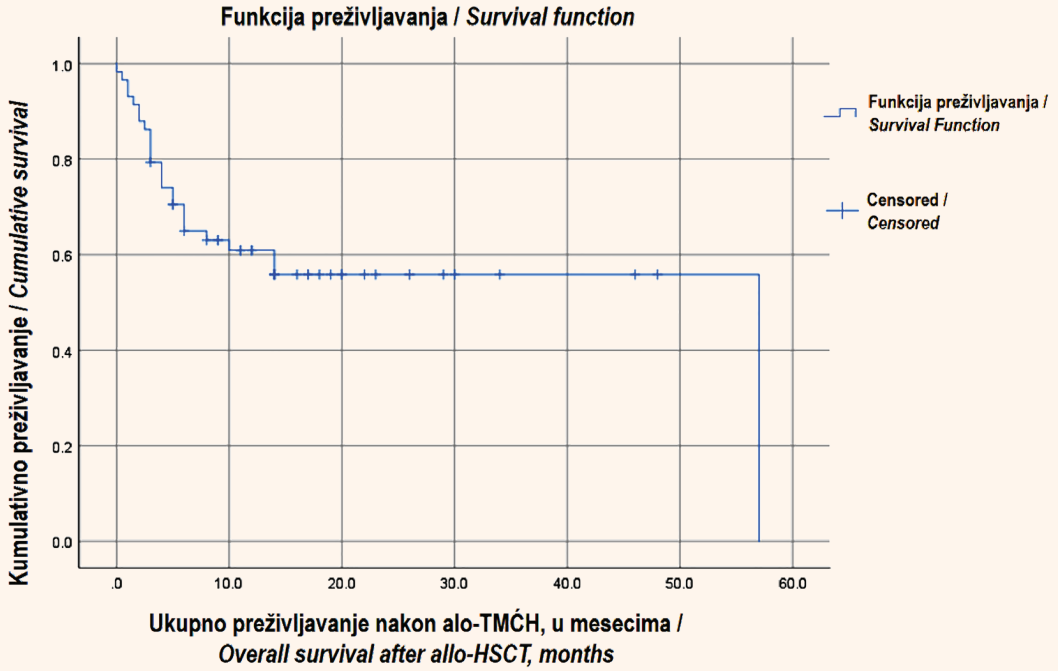
Tabela 3. Distribucija bolesnika lečenih alogenom transplantacijom matičnih ćelija hematopoeze (alo-TMĆH), prema dijagnozama

Table 3. Distribution of patients treated with allogeneic hematopoietic stem cell transplantation (allo-HSCT), by their diagnoses

	n	Procenat / *Percentage* %
ALL	18	31.0
AML	26	44.8
HLL	1	1.7
HL	8	13.8
MDS	1	1.7
MDS/MPN	2	3.4
NHL	2	3.4
Ukupno	58	100.0

ALL – akutna limfoblastna leukemija / *acute lymphoblastic leukemia*
AML – akutna mijeloidna leukemija / *acute myeloid leukemia*
HLL – hronična limfocitna leukemija / *chronic lymphocytic leukemia*
HL – Hočkinov limfom / *Hodgkin's lymphoma*
MDS – mijelodiplastični sindrom / *myelodysplastic syndrome*
MDS/MPN – mijelodisplastični sindrom/mijeloproliferativna neoplazma / *myelodysplastic syndrome/myeloproliferative neoplasm*
NHL – Nehočkinov limfom / *Non-Hodgkin's lymphoma*

After allo-HSCT, the expected survival was 57 months (Appendix IV). On D+30, complete donor chimerism was achieved by 45 patients (77.6%). The conditioning regimens were RIC and MAC, with a dominance of MAC as compared to RIC – MAC: 74.1% (43 of 58 patients); RIC: 25.9% (15 of 58 patients), (Appendix V).



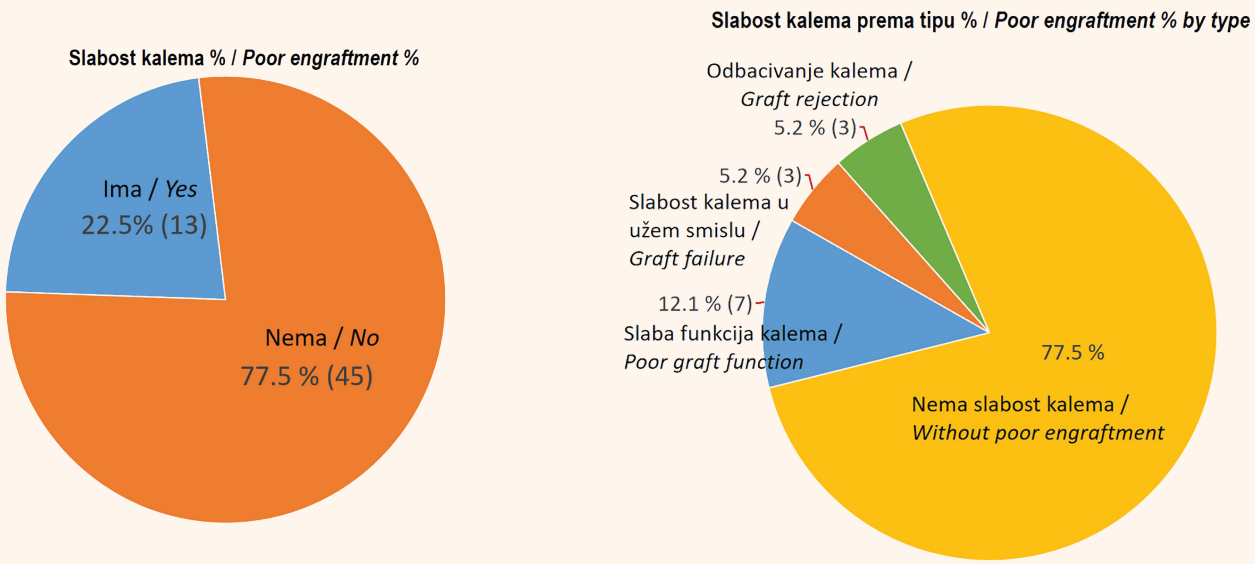
Grafikon 1. Preživljavanje bolesnika nakon alogene transplantacije matičnih ćelija hematopoeze (alo-TMĆH)

Figure 1. Overall survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT)

Tabela 4. Distribucija bolesnika u odnosu na intenzitet kondicionog režima (*MAC* naspram *RIC* režima) i u odnosu na himerizam na D+30

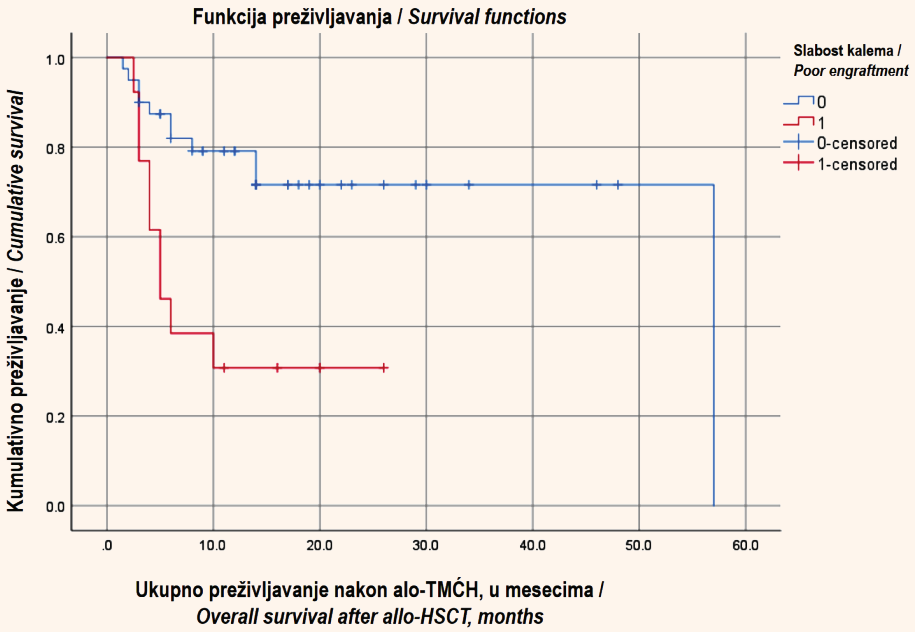
Table 4. Distribution of patients by type of conditioning regimen (MAC vs RIC) and by achievement of donor chimerism on D+30

		n	Procenat / *Percentage*
Himerizam / *Chimerism*	POSTIGNUT / ACHIEVED	50	86.2%
	NIJE POSTIGNUT / NOT ACHIEVED	8	13.8%
Kondicioni režim / *Conditioning regimen*	*MAC*	43	74.1%
	RIC	15	25.9%
UKUPNO / *TOTAL*		58	100



Grafikon 2 i 3. Distribucija pacijenata u odnosu na slabost kalema i u odnosu na sva tri tipa kalema, nakon alogene transplantacije matičnih ćelija hematopoeze (alo-TMĆH)

Figure 2 and 3. Distribution of patients in relation to poor engraftment and in relation to each type of poor engraftment, after allogeneic hematopoietic stem cell transplantation (allo-HSCT)



Grafikon 4. Preživljavanje nakon alogene transplantacije matičnih ćelija hematopoeze (alo-TMĆH), u odnosu na slabost kalema; sa slabošću (1) i bez slabosti kalema (0)

Figure 4. Overall survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT), in relation to poor engraftment (patients who had poor engraftment (1); patients who did not have poor engraftment (0))

jenata bez slabosti kalema iznosilo je 57 meseci, dok je za pacijente koji su razvili slabost kalema ono iznosilo pet meseci, što je bilo statistički značajno manje (p = 0,002), (Prilog VII).

DISKUSIJA

Na osnovu dobijenih rezultata, incidencija slabosti kalema je u našoj studiji iznosila 22,4%, dok se u dostupnoj literaturi ne mogu pronaći podaci o ukupnoj incidenciji slabosti kalema, jer se studije bave svakim tipom slabosti pojedinačno.

Incidencija *PGF*-a se razlikovala od drugih studija i iznosila je 12,1%, što je više nego u studijama koje su sproveli Žao i saradnici (5,18%) i Sun i saradnici (5,6%) [1,4]. Incidencija prijavljena u studijama varira između 5% i 27%, zbog toga što nema tačnih graničnih vrednosti za hemoglobin, trombocite i leukocite, prilikom dijagnostikovanja pancitopenije, koja uz potpuni himerizam, definiše ovaj tip slabosti [2]. Najčešći uzročnik je citomegalovirusna infekcija, koja na direktan (infekcijom ćelija koštane srži) ili indirektan način (infekcijom stromalnih ćelija) inhibira hematopoezu, što se može potkrepiti činjenicom da je svaki pacijent sa *PGF*-om u našoj studiji imao citomegalovirusnu infekciju [7,10]. Neke kliničke studije su pokazale da čak i antivirusna terapija usmerena na CMV, pogotovo ganciklovir, može izazvati mijelosupresiju posle alogene TMĆH [11]. Šimomura i saradnici [5] su pokazali da značajna splenomegalija (≥ 340 cm^3) učestvuje u slabijem engraftmentu trombocita i neutrofila posle transplantacije, kod pacijenata kojima su dijagnostikovani AML i MDS. Takođe, razvoj akutnog *GvHD*-a gradusa II ili većeg, u statistički je značajnoj korelaciji sa *PGF*-om (p = 0,001), što važi i za preopterećenje gvožđem, koje povećava akumulaciju reaktivnog oblika kiseonika (engl. *reactive oxygen species – ROS*), dovodeći do inhibicije hematopoeze i povećavajući rizik od infekcija (služi kao hrana za bakterije i gljivice) [12–14].

Budući da je sve veći broj haploidentičnih alogenih TMĆH, dovodi se u pitanje i uticaj donor specifičnih anti-HLA antitela (DSA) na nastanak *PGF*-a. Sun i saradnici su pokazali značajnu povezanost (incidencija *PGF*-a od 34% kod DSA pozitivnih pacijenata naspram 3,2% kod DSA negativnih pacijenata), dok su Bramanti i saradnici pokazali statistički značajnu povezanost (dva od 26 pacijenata sa *PGF*-om je imalo DSA) [3,4].

S druge strane, incidencija *GF*-a varira između 3,8% i 5,6 %, u zavisnosti od različitih tipova transplantacija [8]. Naša studija je pokazala incidenciju koja odgovara ovom intervalu i ona iznosi 5,2%. Za razliku od *PGF*-a, glavni razlog pojave ovog tipa slabosti je imunski odgovor rezidentnih ćelija recipijenta, zaostalih nakon primene određenog kondicionog režima, na imunohe-

Poor engraftment was found in 13 (22.5%) patients of all those included in the study, while PGF was present in seven patients (12.1% of 58 patients; 53.8% of 13 patients). GF was present in three (5.2% of 58 patients; 23.1% of 13 patients), while GR was also found in three patients (5.2% of 58 patients; 23.1% of 13 patients), (Appendix VI). The expected survival of patients without poor engraftment was 57 months, while patients with poor engraftment had a survival of five months, which is statistically significantly less (p = 0.002), (Appendix VII).

DISCUSSION

Based on the obtained results, the incidence of poor engraftment in our study was 22.4%, while available literature lacks data on the overall incidence of poor engraftment, as previous studies focus on each type of poor engraftment individually.

The incidence of PGF differed from other studies and was 12.1%, which is higher than the studies carried out by Zhao et al. (5.18%) and Sun et al. (5.6%) [1,4]. The incidence reported in studies varies between 5% and 27%, since there are no exact cut-off values for hemoglobin, thrombocytes, and leukocytes, in pancytopenia diagnosis, which, along with complete chimerism, defines this type of poor engraftment [2]. The most common cause of PGF is CMV infection, which directly (through the infection of bone marrow cells) or indirectly (through the infection of stromal cells) inhibits hematopoiesis, which is substantiated by the fact that every patient with PGF in our study had a CMV infection [7,10]. Some clinical studies have shown that even antiviral therapy aimed at CMV, especially ganciclovir, may cause myelosuppression after allo-HSCT [11]. Shimomura et al. [5] showed that significant splenomegaly (≥ 340 cm^3) participates in poor engraftment of thrombocytes and neutrophils after transplantation, in patients diagnosed with AML and MDS. Also, the development of acute GvHD grade II or higher, statistically significantly correlates with PGF (p = 0.001), which is also true of iron overload, which increases the accumulation of reactive oxygen species (ROS), leading to hematopoiesis inhibition and increasing the risk of infection (it serves as food for bacteria and fungi) [12–14].

Bearing in mind the increasing number of haploidentical allogeneic HSCTs, the matter of the impact of donor specific HLA antibodies (DSA) on the occurrence of PGF has also become an issue for consideration. To that effect, Sun et al. reported a significant connection (PGF incidence of 34% in DSA positive patients vs. 3.2% in DSA negative patients), while Bramanti et al. showed a statistically significant link (two out of 26 patients with PGF had DSA) [3,4].

matopoetične ćelije donora. Pokazano je da i donorske citotoksične T ćelije imaju povoljno dejstvo na engraftment, te da i odsustvo istih značajno utiče na povećanu incidenciju *GF*-a [15–17]. Određene studije su pokazale da je kod nemijeloablativnih kondicionih režima, odnosno *RIC*-a, šansa da dođe do lošeg engraftmenta ili pojave *GF*-a znatno veća, dok su u našoj studiji sva tri pacijenta kod kojih je dijagnostikovan *GF* bili u *MAC* režimu [18,19]. Niska doza matičnih ćelija hematopoeze prilikom transplantacije (vrednosti < 2,5 x 10^9), kao i major inkompatibilija u ABO sistemu, značajno utiču na povećanje rizika od *GF*-a, i kod srodne i kod nesrodne transplantacije [18,20]. Budući da je kod naših pacijenata, prilikom transplantacije, prosek doze matičnih ćelija hematopoeze bio 7 x 10^9, te da nije bilo prisustva major inkompatibilije kod sva tri pacijenta, verovatno je uzrok razvoja ove slabosti kalema bio neki od komorbiditeta koji su pacijenti imali.

Kao treći tip slabosti kalema postavlja se *GR*, čija je incidencija bila ista kao kod pacijenata sa *GF*-om i iznosila je 5,2%. Incidencija se ne može uporediti sa ostalim studijama jer se *GR* vodi kao podentitet *GF*-a, zbog toga što je u osnovni njegovog nastanka imunološka reakcija rezidentnih imunih ćelija domaćina. Ovaj tip slabosti može se desiti i kod totalno HLA podudarnih alogenih transplantacija, zbog toga što imunokompatibilne T ćelije koje potiču od domaćina prepoznaju minor histokompatibilne antigene (MiHA) na membranama donorskih ćelija i izazivaju jak imuni odgovor [21]. U alogenoj TMĆH, T regulatorne (T-reg) ćelije, bilo da su od donora ili od recipijenta, igraju bitnu ulogu u engraftmentu. T-reg ćelije domaćina proizvode Il-10 i samim tim potpomažu razvoj nove koštane niše [22]. Nedostatak istih dovodi do *GR*-a posredovanog *natural killer* (*NK)* ćelijama [23,24].

Osim incidencije, preživljavnje dobijeno pomoću Kaplan-Majerove krive predstavlja opšte kumulativno preživljavanje za sve tipove slabosti nakon alogene TMĆH i pokazuje da je očekivano preživljavanje pacijenata sa slabošću kalema bilo pet meseci, što je bilo značajno statistički manje (p = 0,002) u odnosu na pacijente bez slabosti kalema (57 meseci). Ovako slabo preživljavanje možemo uporediti sa slabim opštim preživljavanjem kod svakog tipa pojedinačno. Sun i saradnici [4] su svojom studijom pokazali da je opšte preživljavanje osoba koje su razvile *PGF* bilo značajno manje nego kod pacijenata sa dobrom funkcijom kalema (opšte preživljavanje: 34,6% naspram 82,7%, p < 0,001). U studiji koja se bavila petogodišnjim preživljavanjem osoba sa primarnim *GF-om*, sekundarnim *GF*-om nakon alogene TMĆH, kao i *GR*-om posle alogene transplantacije, preživljavanje je bilo približno isto: 18%, 11% i 13% [22].

On the other hand, the incidence of GF varies between 3.8% and 5.6 %, depending on the different types of transplantation [8]. Our study showed an incidence which was within this interval – it was 5.2%. As opposed to PGF, the main reason for the occurrence of this type of poor engraftment was the immune response of the resident recipient cells, which remained after the implementation of a certain type of conditioning regimen, to the immuno-hematopoietic donor cells. It was demonstrated that donor cytotoxic T cells also have a facilitating effect on engraftment and that their absence significantly effects increased GF incidence [15–17]. Certain studies have shown that, in non-myeloablative conditioning regimens, i.e., RIC regimens, the probability of poor engraftment or of the occurrence of GF is much higher, while in our study, all three patients diagnosed with GF were on the MAC regimen [18,19]. A low dose of hematopoietic stem cells during transplantation (values < 2.5 x 10^9), as well as major incompatibility in the ABO system, significantly affect the increase in the risk of GF, both in related and in unrelated transplantation [18,20]. Since in all our patients, the average dose of hematopoietic stem cells in transplantation was 7 x 10^9, and since there was no major incompatibility in any of the three patients, it is probable that the cause of poor engraftment lay in some of the comorbidities that the patients had.

GR is identified as the third type of poor engraftment. Its incidence was the same as in patients with GF, i.e., 5.2%. The incidence cannot be compared to other studies, due to the fact that GR is identified as a subentity of GF, since the immunological reaction of resident immune host cells is at the core of its development. This type of poor engraftment may occur in totally HLA-matched allogeneic transplantations, due to the fact that incompatible T cells originating from the host recognize minor histocompatible antigens (MiHA) on the donor cell membranes and cause a strong immune response [21]. In allogeneic HSCT, regulatory T cells (T-reg), whether originating from the donor or the recipient, play an important part in engraftment. Host T-reg cells produce Il-10 and thereby facilitate the development of a new bone marrow niche [22]. The lack of these cells leads to GR mediated by natural killer cells [23,24].

In addition to incidence, survival calculated with the Kaplan-Meier curve represents overall cumulative survival for all types of poor engraftment after allogeneic HSCT and shows that the expected survival for patients with poor engraftment was five months, which was statistically significantly shorter (p = 0.002) as compared to patients without poor engraftment (57 months). This poor engraftment can be compared with poor overall survival in each type individually. Sun et al. [4] demon-

ZAKLJUČAK

Slabost kalema može imati za posledicu ozbiljan morbiditet i mortalitet nakon alogene TMĆH, te se sva tri tipa slabosti moraju jasno diferencijalno dijagnostikovati u odnosu na himerizam, jer definisanje tačnog tipa slabosti može značajno da utiče na terapijski pristup pacijentu i konačni ishod. Virusne infekcije, prisustvo *GvHD*-a, nivo gvožđa (serumski feritin kao marker), te splenomegalija, moraju se evaluirati pre dijagnoze slabosti kalema. Takođe treba odrediti nivo DSA pre alogene TMĆH, posebno u slučaju haploidentične transplantacije, da bi se izbegla slabost i postigao uspešan engraftment, pomoću odgovarajućeg kondicionog režima. Veća učestalost slabosti kalema govori u prilog pronalaženju odgovarajuće terapije prevencije ovog aktuelnog problema i poboljšanja njegovog ishoda.

SPISAK SKRAĆENICA

alo-TMĆH – alogena transplantacija matičnih ćelija hematopoeze
GvHD – bolest kalem protiv domaćina (engl. *graft versus host disease*)
PGF – slaba funkcija kalema (engl. *poor graft function*)
GF – slabost kalema u užem smislu (engl. *graft failure*)
GR – odbacivanje kalema (engl. *graft rejection*)
CMV – citomegalovirus
ALL – akutna limfoblastna leukemija
AML – akutna mijeloidna leukemija
HLL – hronična limfocitna leukemija
HL – Hočkinov limfom
NHL – Nehočkinov limfom
SAA – teška aplastična anemija (engl. *severe aplastic anemia*)
MDS/MPN – mijelodisplastični/mijeloproliferativni sindrom
MAC – mijeloablativno kondicioniranje (engl. *myeloablative conditioning*)
RIC – kondicioniranje redukovanog intenziteta (engl. *reduced intensity conditioning*)
DSA – donor specifična antitela
MiHA – minor histokompatibilni antigeni
ROS – reaktivni oblik kiseonika (engl. *reactive oxygen species*)
IST – imunosupresivna terapija
CSA – ciklosporin A
ATG – antitimocitni globulin
T-reg – T regulatorne ćelije
p.o. – *per os*
i.v. – intravenski
TM – telesna masa

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strated in their study that the overall survival in patients who developed PGF was significantly shorter than in patients with a good graft function (overall survival: 34.6% vs. 82.7%; $p < 0.001$). In a study investigating five-year survival in patients with primary GF, secondary GF post allo-HSCT, as well as GR post allo-HSCT, the survival was approximately the same: 18%, 11%, and 13% [22].

CONCLUSION

Poor engraftment can result in serious morbidity and mortality after allogeneic HSCT, which is why all three types of poor engraftment must be clearly differentially diagnosed in relation to chimerism, since the defining of the exact type of poor engraftment can significantly influence the therapeutic approach applied to the patient and the final outcome. Viral infections, the presence of GvHD, the iron level (serum ferritin as marker), and splenomegaly, must be evaluated before the diagnosis of poor engraftment is established. Also, the level of DSA needs to be determined prior to allo-HSCT, especially in haploidentical transplantation, in order to avoid poor engraftment and achieve successful engraftment, with the aid of the appropriate conditioning regimen. A higher incidence of poor engraftment speaks in favor of identifying the appropriate therapy for preventing this ongoing problem and improving its outcome.

LIST OF ABBREVIATIONS AND ACRONYMS

allo-HSCT – allogeneic hematopoietic stem cell transplantation
GvHD – graft versus host disease
PGF – poor graft function
GF – graft failure
GR – graft rejection
CMV – cytomegalovirus
ALL – acute lymphoblastic leukemia
AML – acute myeloid leukemia
HLL – chronic lymphocytic leukemia
HL – Hodgkin's lymphoma
NHL – Non-Hodgkin's lymphoma
SAA – severe aplastic anemia
MDS/MPN – myelodysplastic/myeloproliferative syndrome
MAC – myeloablative conditioning
RIC – reduced intensity conditioning
DSA – donor specific antibodies
MiHA – minor histocompatibility antigens
ROS – reactive oxygen species
IST – immunosuppressive therapy
CSA – cyclosporin A
ATG – anti-thymocyte globulin
T-reg – regulatory T cells
p. o. – *per os*
IV – intravenous
BM – body mass

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