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EDITORIAL

Minimization *vs* tailoring: Where do we stand with personalized immunosuppression during renal transplantation in 2015?

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Abstract

The introduction of novel immunosuppressive agents over the last two decades and the improvement of our diagnostic tools for early detection of antibody-mediated injury offer us an opportunity, if not a mandate, to better match the immunosuppression needs of the individual patients with side effects of the therapy. However, immunosuppressive regimens in the majority of programs remain mostly protocol-driven, with relatively little inter-program heterogeneity in certain areas of the world. Emerging data showing different outcomes with a particular immunosuppressive strategy in populations with varying immunological risks underscore a real potential for "personalized medicine" in renal transplantation. Studies demonstrating marked differences in the adverse-effect profiles of individual drugs including the risk for viral infections, malignancy and renal toxicity call for a paradigm shift away from a "one size fits all" approach to an individually tailored immunosuppressive therapy for renal transplant recipients, assisted by both screening for predictors of graft loss and paying close attention to dose or class-related adverse effects. Our paper explores some of the opportunities during the care of these patients. Potential areas of improvements may include: (1) a thorough assessment of immunological and metabolic risk profile of each renal transplant recipient; (2) screening for predictors of graft loss and early signs of antibody-mediated rejection with donor-specific antibodies, protocol biopsies and proteinuria (including close follow up of adverse effects with dose adjustments or conversions as necessary); and (3) increased awareness of the possible link between poor tolerance of a given drug at a given dose and non-adherence with the prescribed regimen. Altogether, these considerations may enable the most effective use of the drugs we already

have.

Key words: Glucocorticoids; Donor-specific antibodies; Kidney transplantation; Mechanistic (mammalian) target of rapamycin inhibitor; Mycophenolate mofetil; Non-adherence; Calcineurin inhibitor; Sirolimus

Core tip: When managing individual transplant recipients, awareness of potential treatment-induced complications and pre-existing comorbidities may take precedence over excessively rigid adherence to pre-existing pathways. Potential areas of improvement are: (1) a thorough assessment of immunological and metabolic risk profile of each donor recipient; (2) screening for predictors of graft loss and early signs of antibody-mediated rejection with donor-specific antibodies, protocol biopsies and proteinuria (including close follow up of adverse effects with dose adjustments or conversions as necessary); and (3) increased awareness of the possible link between poor tolerance of a given drug at a given dose and non-adherence with the prescribed regimen.

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INTRODUCTION

The introduction of newer immunosuppressive agents, combined with a more widespread use of induction therapy for high risk patients resulted in a substantial reduction of early acute rejections and improved one-year graft survivals; however, these short-term achievements are not matched by similar gains in long-term outcomes of renal allografts[1-3]. With more potent immunosuppression, complications of the therapy evoked a paradigm shift by many clinicians, moving away from further intensification of immunosuppression and to re-focus attention for preventing adverse effects of the immunomodulating therapy such as viral infections, malignancy and inherent renal toxicity[4]. This seemed to have ushered a new era in immunosuppression for renal transplantation: one in which immunosuppressive therapy was strong enough to consider the reduction or elimination of individual immunosuppressive agents associated with long-term toxicities. Thus, the concept of minimization was born. However, minimization seemed to have created yet more controversy: the potential for more rejections with steroid minimization[5,6], increased donor-specific antibody (DSA) development after calcineurin withdrawal[7] and increased graft loss and mortality with mechanistic (mammalian) target of rapamycin (mTOR) inhibitor-based or calcineurin inhibitor (CNI)-free regimens[8,9]. How could we benefit from the fashionable concept of personalization in the field of immunosuppression after renal transplantation? Perhaps, reading the small prints from studies attempting minimization and combining such information with everyday clinical experience might help us to individually tailor immunosuppressive drug combinations. Specifically, while awaiting newer, more potent agents with less toxicity assessing an individual patient's immunological and metabolic risk profile, having appropriate post-transplant screening and attentiveness for adverse events may help us take advantage of what we already have and arrive at the most suitable combination for an individual patient.

ATTEMPTS AT MINIMIZATION: GLUCOCORTICOIDS

The metabolic, bone and cardiovascular side-effects of glucocorticoid hormones, commonly referred to as "steroids" made them a logical target for drug minimization[10]. Given the ever increasing proportion of incident end-stage kidney disease attributable to diabetic nephropathy, glucocorticoid minimization or avoidance maintained steady popularity in the transplant literature[11-14]. Among the more recent studies comparing "steroid-free" regimens to a triple combination of immunosuppressive agents containing glucocorticoids, the FREEDOM trial[5] showed more early acute rejections but a non-inferiority of patient or graft survival in the steroid-free groups. Metabolic side effects known to be associated with glucocorticoid hormones were also reduced. However, in this trial patients with presumed higher immunological risk were excluded, including those receiving allografts from marginal donors or with longer cold ischemia times, recipients with higher panel-reactive antibodies titers, as well as re-transplants. Similar results were obtained in the tacrolimus-based, steroid-free regimens in renal transplantation (ATLAS) trial[6], showing higher acute rejection rates not translating into inferior outcomes but a trend towards better cardiovascular risk profile in the recipients. Furthermore, in the ATLAS trial (a multi-center study of European patients) subjects were at low risk for immunological complications. A retrospective study conducted in the United States on re-transplant patients receiving rabbit-derived anti-thymocyte globulin (rATG) induction therapy[15] showed relatively low rates of acute rejections in both the steroid withdrawal and triple therapy groups. While these and other studies tend to show non-inferiority of steroid-free maintenance regimens in low risk patients - and perhaps a hint that in higher risk patients receiving induction therapy early withdrawal may be safe - it remains unclear whether the improvements in metabolic complications, including new onset diabetes[16], skeletal complications including fracture risk[17] are sufficiently counterbalancing the risk for long-term immunological

complications in these patients. How would tailoring help then? Perhaps the issue of glucocorticoid withdrawal can be used as the most obvious example of personalized immunosuppression. Patients with low immunological risk, or those at a higher immunologic risk but also at risk for metabolic complications could be candidates for glucocorticoid withdrawal, coupled with induction therapy as well as a more intense screening for acute or subclinical rejections, considering the negative impact of acute rejections[18] and increased rates for DSA[19] in this setting. On the other hand, the possibility of increased risk for antibody-mediated rejection after steroid withdrawal in high-risk populations is currently not sufficiently explored. This incomplete state of understanding underscores the importance of close long-term follow-up with increased screening efforts for such patients.

CNI MINIMIZATION: THE FOR AND AGAINST

Since their introduction into maintenance immunosuppression in renal transplant recipients, CNI have greatly contributed to the reduced incidence of acute rejections and improved immediate graft survival[20]. In combination with mycophenolate mofetil and low-dose glucocorticoids, they remain the most popular choice for *de novo* patients in transplant programs throughout North America[21]. However, CNIs are known to have a narrow therapeutic index, require a close monitoring of serum levels and are associated with cumulative renal toxicity. Long-term administration CNI agents may result in renal impairment in both renal[22] and non-renal organ transplant recipients[23], which have led to some disenchantment with CNI in the transplant community[4]. In the background of such functional decline, a distinct histological pattern has been identified with a striped pattern interstitial fibrosis and arterial hyalinosis[24], albeit the specificity of this entity has been challenged recently[25]. The observation that most survival benefits from newer drug combinations, including CNIs is manifested in the first year after transplantation led many to conclude that there may be a dual pattern of graft loss etiology in the post-transplant course after renal transplantation[26]. According to this view, immunological mechanisms may play a prominent role early on manifesting as subclinical rejection on protocol biopsies. Later on, the cumulative toxicity from CNIs may become progressively more significant. This model has led to the development of a dual strategy involving an initial higher intensity immunosuppression with a relative tapering of immunosuppressive drug dosages later on, specifically targeting a lower dose and target levels of CNI during the late transplant course. Nonetheless, an alternative strategy would be the complete elimination of CNI drugs with or without alternative agent(s) introduced. An early study from Australia showed that in patients with low-to-moderate immunological risk, CNIs could be withdrawn within the first year after transplantation with favorable long-term results using graft loss as the primary endpoint[27]. Early studies involving mTOR inhibitors also seemed to have shown promising results as discussed in the chapter below. However, this strategy has been recently challenged by newer studies taking advantage of recent developments in the diagnostic armamentarium for antibody-mediated rejection. Renal allograft biopsies taken "for cause" in North American transplant centers[28] showed that humoral rejection may be the single most important etiology behind a declining graft function. In this particular series, calcineurin toxicity seemed much less prominent than previously reported. The same study drew attention to the significance of non-adherence to immunosuppressive regimens, possibly enhancing the role played by immunological mechanisms in these patients. Under such circumstances, inadequate immunosuppression due to non-adherence may substantially contribute to graft loss. In the opinion of the authors of this paper, this is a crucial point which may not be emphasized enough for daily practice transplant medicine.

The diagnostic accuracy of CNI–toxicity[25] and the very notion that progressive decline in graft function may be associated with chronic calcineurin toxicity has also been called in question by some[29] arguing that in the absence of DSA and serum complement factor 4, d-fragment (C4d) staining the histological diagnosis of "calcineurine inhibitor toxicity" carries a relatively good prognosis. Understanding the relative importance of these contributing mechanisms is not at all trivial If CNI toxicity is relatively common even at dosages currently in use, then CNI minimization is a valid strategy aiming at preserving functional renal parenchyma and maintaining longevity of grafts. If, on the other hand, antibody-mediated mechanisms play a more prominent role in patients with higher immunological risk, CNI minimization may be counter-productive by lowering anti-rejection defense at a time when such is most needed. This state of affairs clearly points to the importance of developing screening tools to identify patients at higher risk for antibody-mediated rejection. This would allow us tailoring in lieu of minimization: those more at risk for antibody-mediated immune mechanisms would be maintained on relatively higher doses of CNIs with or without low dose glucocorticoid hormones, while those at low risk may be more suitable candidates for calcineurin minimization or withdrawal. Do we have these screening tools in 2015? If so, how should we use them?

INDIVIDUALIZATION: RISK PROFILE AND SCREENING TOOLS

It has been well recognized that a number of donor and recipient-related factors as well as factors associated with preservation injury may influence the risk of

graft loss after renal transplantation. In fact, a scoring system predicting graft loss has been developed on such basis[30]. It is logical to assume that patients with higher risk for graft loss may need more potent immunosuppression in the early post-transplant period with induction therapy and a CNI-based triple combination. Keenly aware of the cumulative toxicity associated with such therapies, including viral infections [cytomegalovirus (CMV), polyoma-BK virus, Epstein-Barr virus infections], malignancy and renal toxicity, calcineurin minimization or withdrawal with or without replacement of CNIs by alternative agents have been attempted both early and late after transplantation[27,31-36]. These studies showed divergent results: some showing benefit with better renal function after CNI minimization[27,31,33-35], while others failing to show such favorable outcomes[34,36]. Overall, the main factors predicting a favorable outcome are well-preserved initial renal function (glomerular filtration rate > 40 mL/min per 1.73 m^2), lower levels of proteinuria (< 1 g/d), absence of previous acute or subclinical rejection and no subsequent appearance of donor-specific anti-human leukocyte antigen antibodies[36,37]. A recent report on 5-year outcomes of patients converted to everolimus four and half months after transplantation under the auspices of the ZEUS trial[38] confirms the safety and tolerability of such an approach with a low mortality rate (< 3%), a fairly high rate of patients remaining on mTOR inhibitor after 5 years (62.6%) and an adverse event rate not significantly different from the control arm (*i.e.*, patients remaining on cyclosporine). An increased incidence of mild acute rejections did not seem to translate into worse function or graft loss; on the contrary eGFR remained higher in the everolimus group (estimated GFR 66.2 mL/min per 1.73 m^2 with everolimus *vs* 60.9 mL/min per 1.73 m^2 with cyclosporine-A; mean difference 5.3 mL/min per 1.73 m^2 in favor of everolimus in intent-to-treat population). While these results are encouraging suggesting that mTOR inhibitors may represent a viable alternative to CNIs in certain low risk patients, concerns for increased de novo DSA production and proteinuria remain, particularly when an mTOR-based regimen is compared to the slightly more contemporary tacrolimus-based regimens.

In order to optimize the decision making process to individually tailor immunosuppression according to the patient's actual needs, we should take full advantage of the screening tools already available to identify cases with ongoing subclinical antibody-mediated injury in the renal graft. Protocol biopsy has been shown to be a useful tool in identifying patients with subclinical rejection early in the post-transplant course[26]. The recognition that subclinical rejection did appear in a substantial number of patients within the first year after kidney transplantation may be instrumental in guiding our therapy further. Histological lesions found on protocol biopsies may be even more predictive when coupled with the presence of donor-specific antibodies. It has been shown that the combined appearance of C4d staining and DSA is associated with a substantially worse graft survival when compared to either presenting alone. The presence of DSA, nonetheless, appears to be an independent predictor of graft loss[39,40]. Moreover, the appearance of DSA is associated with non-adherence and prior rejections[39] as well as an mTOR-based immunosuppression compared to CNI use[7]. Though DSA monitoring has recently been introduced into routine clinical practice, there are no clear guidelines on how to use this information. With the presence of extremely sensitive techniques to identify DSA at low titers in otherwise completely asymptomatic and stable patients, what should be the next logical step after identifying *de novo* appearance of DSA? Perhaps the presence of C4d or subclinical rejections on protocol biopsies or the presence of progressive and otherwise unexplained albuminuria may strengthen the case for a more aggressive treatment strategy in these patients. Persistent proteinuria was part of the early definitions of chronic kidney disease[41] and it has long been known to be an important cardiovascular and renal predictor in both diabetic and non-diabetic renal disease. In addition, proteinuria is common after renal transplantation and it has been identified as an important predictor for graft loss, adverse cardiovascular events and increased overall mortality in renal transplant recipients[42]. It is also predictive of adverse outcomes at low levels when presenting early after transplantation[43]. Moreover, proteinuria is a consistent feature in acute rejection and is one of the clinical hallmarks in transplant glomerulopathy. Furthermore, a link seems to exist between appearance of DSA and proteinuria, whereas proteinuria seems to precede the appearance of DSA and appears to be an important factor predicting rapid decline of graft function[44]. Additional efforts to explore the relationship between *de novo* appearance of DSA and low-level proteinuria in otherwise clinically stable patients may prove to be useful in the clinical decision-making process for such patients. In the absence of definitive studies on this subject, close monitoring of proteinuria may be advisable in all patients. Persistent proteinuria even at low absolute levels should alert one to the possibility that a subclinical antibody-mediated process may be at work. In such patients, minimizing or withdrawing CNIs or steroids may prove to be deleterious.

MINIMIZATION AND THE ROLE OF MTOR INHIBITORS

The early promise of mTOR inhibitors was that they could potentially provide some relief from the long-term toxicities of CNIs[45]. Antiproliferative, antitumoral[46-48] and antiviral effects, including effects against CMV[45,49], polyoma-BK[50] and other viruses[47] coupled with a lack of nephrotoxicity[45] appeared attractive properties and fit right into the strategy of CNI minimization or

withdrawal at either an early or later time point after transplantation. It soon became apparent, nonetheless, that the role for mTOR inhibitors may be limited in the setting when a certain amount of cumulative damage due to CNI toxicity has already been reached. In a 24-mo efficacy and safety conversion trial from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients trial showed that no apparent graft survival benefit could be achieved after substitution of CNIs for mTOR inhibitors in patients with already low GFR or substantial proteinuria[36]. However, multiple trials suggested that earlier introduction of mTOR inhibitors coupled with dose reduction (*i.e.*, an mTOR/calcineurin combination)[51] or conversion to an mTOR inhibitor with complete CNI withdrawal[32,33,35,49,52] may be beneficial in terms of preserving renal function and lowering the incidence of both CMV infections[53], polyoma BK virus infection[50] and malignancy[54-56]. However, concerns have been raised about such strategies due to a number of emerging issues associated with mTOR inhibitors including non-adherence to protocols[34], increased mortality and graft loss[8,9,35], worsening proteinuria[35] and increased incidence of DSA[7]. Partly due to these considerations and perhaps even to a larger extent due to an unfavorable adverse effect profile associated with mTOR inhibitors, the use of this strategy has sharply declined in North America[21]. This, in turn, gave rise to a dichotomy between the United States and other developed regions in terms of immunosuppressive strategies, a pattern curiously reminiscent of what we had observed during international comparisons of hemodialysis practices[57]. Strangely, a dichotomy also seems to exist in terms of graft survival[58], a phenomenon certainly not yet sufficiently analyzed. While in the United States most programs appear to favor a more homogeneous approach with induction therapy, tacrolimus, mycophenolate with or without maintenance steroids[21], in Europe several programs use mTOR inhibitor-based combinations reporting more favorable clinical outcomes, particularly in low risk patients[37]. What may lie behind such differences? Due to the lack of reliable data, the authors are forced to rely on their own experiences. While there may clearly be important differences in the immunological risk profiles and perhaps in drug metabolism in different patient populations, there also seems to be important regional differences in mTOR inhibitor dosing. North American studies reporting higher mortality and graft loss reported mTOR inhibitor dosages and levels substantially higher[9] than we have seen in some European programs and these higher dosages were, in turn, associated with more frequent adverse effects and non-adherence to mTOR-based regimens. This latter point cannot be emphasized sufficiently. Lower adherence may be associated with graft loss and antibody-mediated humoral mechanisms[28] and in many instances might be due to higher-than-tolerable dosing in an important minority of the patients. This might suggest that such patients could benefit from dose reduction. However, such a strategy is possible only when a sufficiently close follow up is in place to uncover tolerability-limiting adverse effects of a particular immunosuppressive agent.

TAILORING: MAKING USE OF WHAT WE HAVE

Even though we have great promise from newer immunosuppressive agents, an individualized use of drugs we already have available may enlarge our therapeutic horizon further. This presupposes two factors: (1) a thorough evaluation of all risks, including immunological risk due to donor, preservation or recipient-related factors and the recipient's metabolic risk for new onset diabetes, hyperlipidemia and weight gain; (2) screening for circulating donor-specific antibodies with or without protocol biopsies or with more conventional renal predictors including proteinuria. Additionally, during chronic follow-up, the physician should carefully screen for adverse effects limiting tolerability of a specific drug class, keeping in mind that many of these side-effects may be dose-dependent. For *de novo* patients with high immunological risk, the current practice of giving induction therapy with a lymphocyte-depleting agent and a CNI-based triple therapy seems a logical choice. However, in patients with lower immunological risk the treatment regimens could be more diversified. For instance, in patients at higher risk for CMV or BK viral infections, or those not tolerating inosine monophosphate dehydrogenase (IMPDH) inhibitors (inhibitors of lymphocyte *de novo* purine nucleotide biosynthesis; *i.e.*, mycophenolate mofetil and mycophenolic acid) in sufficient dosages, the synergistic effects of a calcineurin-mTOR inhibitor could be utilized to keep both drugs at a lower dosage. Clinical experience suggests - at least in European patients, - that a relatively low "combined target level" of 7-10 for tacrolimus-mTOR combination (whole blood levels of tacrolimus and mTOR inhibitor summed up together, both expressed in ng/mL) may provide sufficient immunosuppression while avoiding many of the adverse reactions associated with higher targets used historically. For those at risk for calcineurin-associated adverse effects including malignancy, mTOR conversion may be logical choice. Often such patients may not require high mTOR dosages and tolerate such regimens reasonably well. Patients with *de novo* appearance of DSA, especially combined with rising levels of proteinuria may benefit from a relatively higher level of maintenance immunosuppression, and preferentially CNI-based one. Conversely, patients on CNI-minimized regimens or after CNI withdrawal may benefit from close monitoring for DSA and proteinuria, given the data for a higher incidence of de novo DSA appearance in such patients[7]. Patients at higher risk for metabolic complications, such as new onset diabetes, may benefit from an IMPDH-

based immunosuppressive regimen provided that a relatively high dose is well tolerated. Steroid sparing may be important in such patients, but this may need to be counterbalanced against the higher risk for acute rejections[5,6] that may or may not translate into higher antibody-mediated mechanisms later in the transplant course.

Emerging data on costimulation blockade-based regimens provide promise that a new alternative to CNI-based regimens may become available in centers that are able to afford the high costs associated with belatacept. Reports on five-year outcome data do indicate that despite a higher incidence of early acute rejections renal function and patient safety are maintained with belatacept and the incidence of post-transplant lymphoproliferative disorder remains acceptable, especially in patients that are seropositive for Ebstein-Bar virus at the time of transplantation[59,60]. Conversion from CNI to belatacept also appears to be possible without evidence for inferiority in terms of patient survival or graft outcomes[61]. Should belatacept become more accessible in the future, enough clinical experience may accumulate to define a role for this promising agent in patients with appropriate risk and safety profiles.

Finally, with emerging data emphasizing the importance of non-adherence[28], we should keep in mind close monitoring for adverse reactions. Early detection of a compliance-endangering side effect gives us the opportunity to tailor dose or to choose an alternative drug to accommodate individual susceptibilities or side effects.

CONCLUSION

In practice of clinical medicine, we often have to make the best decision based on less-than-complete information or in patients with multiple co-existing comorbidities; therefore, the concept of "evidence-based medicine" itself becomes a contradiction. Accordingly, when managing an individual side effect, complications and co-morbidities may take precedence over excessively rigid adherence to pre-existing pathways. Perhaps the time has come to abandon the "one size fits all" approach and to go beyond using rigid protocols in choosing the optimal immunosuppressive regimen for an individual patient. Potential areas of considerations are: (1) a thorough assessment of immunological and metabolic risk profile of each recipient; (2) screening for predictors of graft loss and early signs of antibody-mediated rejection with DSA, protocol biopsies and proteinuria (including close follow up of adverse effects with dose adjustments or conversions as necessary); and (3) increased awareness of the possible link between poor tolerance of a given drug at a given dose and non-adherence with the prescribed regimen. Altogether, these considerations may broaden our therapeutic horizon and makes possible the most effective use of the drugs we already have.

REFERENCES

1 **Lodhi SA**, Meier-Kriesche HU. Kidney allograft survival: the long and short of it. *Nephrol Dial Transplant* 2011; **26**: 15-17 [PMID: 21177522 DOI: 10.1093/ndt/gfq730]

2 **Meier-Kriesche HU**, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; **4**: 1289-1295 [PMID: 15268730 DOI: 10.1111/j.1600-6143.2004.00515.x]

3 **Chang SH**, Russ GR, Chadban SJ, Campbell SB, McDonald SP. Trends in kidney transplantation in Australia and New Zealand, 1993-2004. *Transplantation* 2007; **84**: 611-618 [PMID: 17876274 DOI: 10.1097/01.tp.0000280553.23898.ef]

4 **Salvadori M**, Bertoni E. Is it time to give up with calcineurin inhibitors in kidney transplantation? *World J Transplant* 2013; **3**: 7-25 [PMID: 24175203 DOI: 10.5500/wjt.v3.i2.7]

5 **Vincenti F**, Schena FP, Paraskevas S, Hauser IA, Walker RG, Grinyo J. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. *Am J Transplant* 2008; **8**: 307-316 [PMID: 18211506 DOI: 10.1111/j.1600-6143.2007.02057.x]

6 **Krämer BK**, Klinger M, Vítko Š, Glyda M, Midtvedt K, Stefoni S, Citterio F, Pietruck F, Squifflet JP, Segoloni G, Krüger B, Sperschneider H, Banas B, Bäckman L, Weber M, Carmellini M, Perner F, Claesson K, Marcinkowski W, Ostrowski M, Senatorski G, Nordström J, Salmela K. Tacrolimus-based, steroid-free regimens in renal transplantation: 3-year follow-up of the ATLAS trial. *Transplantation* 2012; **94**: 492-498 [PMID: 22858806 DOI: 10.1097/TP.0b013e31825c1d6c]

7 **Liefeldt L**, Brakemeier S, Glander P, Waiser J, Lachmann N, Schönemann C, Zukunft B, Illigens P, Schmidt D, Wu K, Rudolph B, Neumayer HH, Budde K. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. *Am J Transplant* 2012; **12**: 1192-1198 [PMID: 22300538 DOI: 10.1111/j.1600-6143.2011.03961.x]

8 **Isakova T**, Xie H, Messinger S, Cortazar F, Scialla JJ, Guerra G, Contreras G, Roth D, Burke GW, Molnar MZ, Mucsi I, Wolf M. Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. *Am J Transplant* 2013; **13**: 100-110 [PMID: 23025566 DOI: 10.1111/j.1600-6143.2012.04281.x]

9 **Guerra G**, Ciancio G, Gaynor JJ, Zarak A, Brown R, Hanson L, Sageshima J, Roth D, Chen L, Kupin W, Tueros L, Ruiz P, Livingstone AS, Burke GW. Randomized trial of immunosuppressive regimens in renal transplantation. *J Am Soc Nephrol* 2011; **22**: 1758-1768 [PMID: 21807891]

10 **Vincenti F**. Interleukin-2 receptor antagonists and aggressive steroid minimization strategies for kidney transplant patients. *Transpl Int* 2004; **17**: 395-401 [PMID: 15365604 DOI: 10.1111/j.1432-2277.2004.tb00462.x]

11 **Vincenti F**, Monaco A, Grinyo J, Kinkhabwala M, Roza A. Multicenter randomized prospective trial of steroid withdrawal in renal transplant recipients receiving basiliximab, cyclosporine microemulsion and mycophenolate mofetil. *Am J Transplant* 2003; **3**: 306-311 [PMID: 12614286 DOI: 10.1034/j.1600-6143.2003.00005.x]

12 **Rostaing L**, Cantarovich D, Mourad G, Budde K, Rigotti P, Mariat C, Margreiter R, Capdevilla L, Lang P, Vialtel P, Ortuño-Mirete J, Charpentier B, Legendre C, Sanchez-Plumed J, Oppenheimer F, Kessler M. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807-814 [PMID: 15818323 DOI: 10.1097/01.TP.0000154915.20524.0A]

13 **Kumar MS**, Xiao SG, Fyfe B, Sierka D, Heifets M, Moritz MJ, Saeed MI, Kumar A. Steroid avoidance in renal transplantation using basiliximab induction, cyclosporine-based immunosuppression and protocol biopsies. *Clin Transplant* 2005; **19**: 61-69 [PMID: 15659136 DOI: 10.1111/j.1399-0012.2004.00298.x]

14 **Kandaswamy R**, Melancon JK, Dunn T, Tan M, Casingal V, Humar A, Payne WD, Gruessner RW, Dunn DL, Najarian JS, Sutherland

DE, Gillingham KJ, Matas AJ. A prospective randomized trial of steroid-free maintenance regimens in kidney transplant recipients--an interim analysis. *Am J Transplant* 2005; **5**: 1529-1536 [PMID: 15888064 DOI: 10.1111/j.1600-6143.2005.00885.x]

15 **Mujtaba MA**, Taber TE, Goggins WC, Yaqub MS, Mishler DP, Milgrom ML, Fridell JA, Lobashevsky A, Powelson JA, Sharfuddin AA. Early steroid withdrawal in repeat kidney transplantation. *Clin J Am Soc Nephrol* 2011; **6**: 404-411 [PMID: 21051751 DOI: 10.2215/CJN.05110610]

16 **Luan FL**, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. *Transplantation* 2011; **91**: 334-341 [PMID: 21242885 DOI: 10.1097/TP.0b013e318203c25f]

17 **Nikkel LE**, Mohan S, Zhang A, McMahon DJ, Boutroy S, Dube G, Tanriover B, Cohen D, Ratner L, Hollenbeak CS, Leonard MB, Shane E, Nickolas TL. Reduced fracture risk with early corticosteroid withdrawal after kidney transplant. *Am J Transplant* 2012; **12**: 649-659 [PMID: 22151430 DOI: 10.1111/j.1600-6143.2011.03872.x]

18 **Heilman RL**, Nijim S, Chakkera HA, Devarapalli Y, Moss AA, Mulligan DC, Mazur MJ, Hamawi K, Williams JW, Reddy KS. Impact of acute rejection on kidney allograft outcomes in recipients on rapid steroid withdrawal. *J Transplant* 2011; **2011**: 583981 [PMID: 21647349]

19 **Hoshino J**, Kaneku H, Everly MJ, Greenland S, Terasaki PI. Using donor-specific antibodies to monitor the need for immunosuppression. *Transplantation* 2012; **93**: 1173-1178 [PMID: 22592887 DOI: 10.1097/TP.0b013e31824f3d7c]

20 **Casey MJ**, Meier-Kriesche HU. Calcineurin inhibitors in kidney transplantation: friend or foe? *Curr Opin Nephrol Hypertens* 2011; **20**: 610-615 [PMID: 21885969 DOI: 10.1097/MNH.0b013e32834b4343]

21 USRDS 2012 Annual Data Report. Chapter 7, Figure 7.27, 2015 [Accessed January 22, 2015] Available from: URL: http://www.usrds.org/2012/view/v2_07.aspx

22 **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004; **78**: 557-565 [PMID: 15446315 DOI: 10.1097/01.TP.00001 28636.70499.6E]

23 **Bloom RD**, Reese PP. Chronic kidney disease after nonrenal solid-organ transplantation. *J Am Soc Nephrol* 2007; **18**: 3031-3041 [PMID: 18039925 DOI: 10.1681/ASN.2007040394]

24 **Liptak P**, Ivanyi B. Primer: Histopathology of calcineurin-inhibitor toxicity in renal allografts. *Nat Clin Pract Nephrol* 2006; **2**: 398-404; quiz following 404 [PMID: 16932468 DOI: 10.1038/ncpneph0225]

25 **Mengel M**, Mihatsch M, Halloran PF. Histological characteristics of calcineurin inhibitor toxicity--there is no such thing as specificity! *Am J Transplant* 2011; **11**: 2549-2550 [PMID: 21883916 DOI: 10.1111/j.1600-6143.2011.03719.x]

26 **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326-2333 [PMID: 14668458 DOI: 10.1056/NEJMoa020009]

27 **Gallagher MP**, Hall B, Craig J, Berry G, Tiller DJ, Eris J. A randomized controlled trial of cyclosporine withdrawal in renal-transplant recipients: 15-year results. *Transplantation* 2004; **78**: 1653-1660 [PMID: 15591955 DOI: 10.1097/01.TP.0000144181.47045.FE]

28 **Sellarés J**, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, Hidalgo LG, Famulski K, Matas A, Halloran PF. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388-399 [PMID: 22081892 DOI: 10.1111/j.1600-6143.2011.03840.x]

29 **Matas AJ**. Chronic progressive calcineurin nephrotoxicity: an overstated concept. *Am J Transplant* 2011; **11**: 687-692 [PMID: 21446973 DOI: 10.1111/j.1600-6143.2011.03505.x]

30 **Schold JD**, Kaplan B, Baliga RS, Meier-Kriesche HU. The broad spectrum of quality in deceased donor kidneys. *Am J Transplant* 2005; **5**: 757-765 [PMID: 15760399 DOI: 10.1111/j.1600-6143.2005.00770.x]

31 **Legendre C**, Brault Y, Morales JM, Oberbauer R, Altieri P, Riad H, Mahony J, Messina M, Pussell B, Martínez JG, Lelong M, Burke JT, Neylan JF. Factors influencing glomerular filtration rate in renal transplantation after cyclosporine withdrawal using sirolimus-based therapy: a multivariate analysis of results at five years. *Clin Transplant* 2007; **21**: 330-336 [PMID: 17488381 DOI: 10.1111/j.1399-0012.2007.00645.x]

32 **Weir MR**, Mulgaonkar S, Chan L, Shidban H, Waid TH, Preston D, Kalil RN, Pearson TC. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. *Kidney Int* 2011; **79**: 897-907 [PMID: 21191361 DOI: 10.1038/ki.2010.492]

33 **Lebranchu Y**, Thierry A, Toupance O, Westeel PF, Etienne I, Thervet E, Moulin B, Frouget T, Le Meur Y, Glotz D, Heng AE, Onno C, Buchler M, Girardot-Seguin S, Hurault de Ligny B. Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant* 2009; **9**: 1115-1123 [PMID: 19422337 DOI: 10.1111/j.1600-6143.2009.02615.x]

34 **Holdaas H**, Rostaing L, Serón D, Cole E, Chapman J, Fellstrøm B, Strom EH, Jardine A, Midtvedt K, Machein U, Ulbricht B, Karpov A, O'Connell PJ. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation* 2011; **92**: 410-418 [PMID: 21697773 DOI: 10.1097/TP.0b013e318224c12d]

35 **Budde K**, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, Kramer S, Fischer W, Gschaidmeier H, Pietruck F. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 2011; **377**: 837-847 [PMID: 21334736 DOI: 10.1016/S0140-6736(10)62318-5]

36 **Schena FP**, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC, Campistol JM, Racusen L, Polinsky MS, Goldberg-Alberts R, Li H, Scarola J, Neylan JF. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation* 2009; **87**: 233-242 [PMID: 19155978 DOI: 10.1097/TP.0b013e3181927a41]

37 **Gatault P**, Lebranchu Y. Conversion to mTOR-inhibitor-based immunosuppression: which patients and when? *Transplant Res* 2013; **2**: S3 [PMID: 24565231 DOI: 10.1186/2047-1440-2-S1-S3]

38 **Budde K**, Lehner F, Sommerer C, Reinke P, Arns W, Eisenberger U, Wüthrich RP, Mühlfeld A, Heller K, Porstner M, Veit J, Paulus EM, Witzke O. Five-year outcomes in kidney transplant patients converted from cyclosporine to everolimus: the randomized ZEUS study. *Am J Transplant* 2015; **15**: 119-128 [PMID: 25521535 DOI: 10.1111/ajt.12952]

39 **Wiebe C**, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, Goldberg A, Birk PE, Rush DN, Nickerson PW. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant* 2012; **12**: 1157-1167 [PMID: 22429309 DOI: 10.1111/j.1600-6143.2012.04013.x]

40 **Kokko KE**, Colvin RB. Below the waterline -- the danger of de novo donor-specific HLA antibodies. *Am J Transplant* 2012; **12**: 1077-1078 [PMID: 22537262 DOI: 10.1111/j.1600-6143.2012.04016.x]

41 **Coresh J**, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003; **41**: 1-12 [PMID: 12500213 DOI: 10.1053/ajkd.2003.50007]

42 **Shamseddin MK**, Knoll GA. Posttransplantation proteinuria: an approach to diagnosis and management. *Clin J Am Soc Nephrol* 2011; **6**: 1786-1793 [PMID: 21734095 DOI: 10.2215/CJN.01310211]

43 **Hernández D**, Pérez G, Marrero D, Porrini E, Rufino M, González-

Posada JM, Delgado P, Torres A. Early association of low-grade albuminuria and allograft dysfunction predicts renal transplant outcomes. *Transplantation* 2012; **93**: 297-303 [PMID: 22228419 DOI: 10.1097/TP.0b013e31823ec0a7]

44 **Fotheringham J**, Angel C, Goodwin J, Harmer AW, McKane WS. Natural history of proteinuria in renal transplant recipients developing de novo human leukocyte antigen antibodies. *Transplantation* 2011; **91**: 991-996 [PMID: 21519315 DOI: 10.1097/TP.0b013e3182126ed0]

45 **Nashan B**, Curtis J, Ponticelli C, Mourad G, Jaffe J, Haas T. Everolimus and reduced-exposure cyclosporine in de novo renal-transplant recipients: a three-year phase II, randomized, multicenter, open-label study. *Transplantation* 2004; **78**: 1332-1340 [PMID: 15548972 DOI: 10.1097/01.TP.0000140486.97461.49]

46 **Sehgal SN**. Sirolimus: its discovery, biological properties, and mechanism of action. *Transplant Proc* 2003; **35**: 7S-14S [PMID: 12742462 DOI: 10.1016/S0041-1345(03)00211-2]

47 **Campistol JM**, Gutierrez-Dalmau A, Torregrosa JV. Conversion to sirolimus: a successful treatment for posttransplantation Kaposi's sarcoma. *Transplantation* 2004; **77**: 760-762 [PMID: 15021843 DOI: 10.1097/01.TP.0000115344.18025.0B]

48 **Salvadori M**. Antineoplastic effects of mammalian target of rapamycine inhibitors. *World J Transplant* 2012; **2**: 74-83 [PMID: 24175199 DOI: 10.5500/wjt.v2.i5.74]

49 **Lim W**, Eris J, Kanellis J, Pussell B, Wiid Z, Witcombe D, Russ G. [SA588] Conversion from calcineurin-inhibitor to mammalian target of rapamycin inhibitors for maintenance immunosuppression in kidney transplant recipients: a systematic review and meta-analysis of randomized trials Paper presented at: ISN World Congress of Nephrology; 31 May-4 June 2013, Hong Kong, 2013

50 **Suwelack B**, Malyar V, Koch M, Sester M, Sommerer C. The influence of immunosuppressive agents on BK virus risk following kidney transplantation, and implications for choice of regimen. *Transplant Rev* (Orlando) 2012; **26**: 201-211 [PMID: 21940156 DOI: 10.1016/j.trre.2011.05.002]

51 **Tedesco Silva H**, Cibrik D, Johnston T, Lackova E, Mange K, Panis C, Walker R, Wang Z, Zibari G, Kim YS. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. *Am J Transplant* 2010; **10**: 1401-1413 [PMID: 20455882 DOI: 10.1111/j.1600-6143.2010.03129.x]

52 **Lebranchu Y**, Thierry A, Thervet E, Büchler M, Etienne I, Westeel PF, Hurault de Ligny B, Moulin B, Rérolle JP, Frouget T, Girardot-Seguin S, Toupance O. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the Postconcept study. *Am J Transplant* 2011; **11**: 1665-1675 [PMID: 21797975 DOI: 10.1111/j.1600-6143.2011.03637.x]

53 **Andrassy J**, Hoffmann VS, Rentsch M, Stangl M, Habicht A, Meiser B, Fischereder M, Jauch KW, Guba M. Is cytomegalovirus prophylaxis dispensable in patients receiving an mTOR inhibitor-based immunosuppression? a systematic review and meta-analysis. *Transplantation* 2012; **94**: 1208-1217 [PMID: 23269449 DOI: 10.1097/TP.0b013e3182708e56]

54 **Kauffman HM**, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation* 2005; **80**: 883-889 [PMID: 16249734 DOI: 10.1097/01.TP.0000184006.43152.8D]

55 **Campistol JM**, Eris J, Oberbauer R, Friend P, Hutchison B, Morales JM, Claesson K, Stallone G, Russ G, Rostaing L, Kreis H, Burke JT, Brault Y, Scarola JA, Neylan JF. Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J Am Soc Nephrol* 2006; **17**: 581-589 [PMID: 16434506 DOI: 10.1681/ASN.2005090993]

56 **Alberú J**, Pascoe MD, Campistol JM, Schena FP, Rial Mdel C, Polinsky M, Neylan JF, Korth-Bradley J, Goldberg-Alberts R, Maller ES. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurin inhibitor-free immunotherapy: 24-month results from the CONVERT trial. *Transplantation* 2011; **92**: 303-310 [PMID: 21792049 DOI: 10.1097/TP.0b013e3182247ae2]

57 **Zsom L**, Zsom M, Fulop T, Flessner MF. Treatment time, chronic inflammation, and hemodynamic stability: the overlooked parameters in hemodialysis quantification. *Semin Dial* 2008; **21**: 395-400 [PMID: 18945325 DOI: 10.1111/j.1525-139X.2008.00488.x]

58 **Gondos A**, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation* 2013; **95**: 267-274 [PMID: 23060279 DOI: 10.1097/TP.0b013e3182708ea8]

59 **Rostaing L**, Vincenti F, Grinyó J, Rice KM, Bresnahan B, Steinberg S, Gang S, Gaite LE, Moal MC, Mondragón-Ramirez GA, Kothari J, Pupim L, Larsen CP. Long-term belatacept exposure maintains efficacy and safety at 5 years: results from the long-term extension of the BENEFIT study. *Am J Transplant* 2013; **13**: 2875-2883 [PMID: 24047110 DOI: 10.1111/ajt.12460]

60 **Charpentier B**, Medina Pestana JO, Del C Rial M, Rostaing L, Grinyó J, Vanrenterghem Y, Matas A, Zhang R, Mühlbacher F, Pupim L, Florman S. Long-term exposure to belatacept in recipients of extended criteria donor kidneys. *Am J Transplant* 2013; **13**: 2884-2891 [PMID: 24103072 DOI: 10.1111/ajt.12459]

61 **Rostaing L**, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, Steinberg S, Vincenti F, Shi R, Di Russo G, Thomas D, Grinyó J. Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized phase II study. *Clin J Am Soc Nephrol* 2011; **6**: 430-439 [PMID: 21051752 DOI: 10.2215/CJN.05840710]

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Recent advances in post autologous transplantation maintenance therapies in B-cell non-Hodgkin lymphomas

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Abstract

Lymphomas constitute the second most common indication for high dose therapy (HDT) followed by autologous hematopoietic cell transplantation (auto-HCT). The intent of administering HDT in these heterogeneous disorders varies from cure (*e.g.*, in relapsed aggressive lymphomas) to disease control (*e.g.*, most indolent lymphomas). Regardless of the underlying histology or remission status at transplantation, disease relapse remains the number one cause of post auto-HCT therapy failure and mortality. The last decade has seen a proliferation of clinical studies looking at prevention of post auto-HCT therapy failure with various maintenance strategies. The benefit of such therapies is in turn dependent on disease histology and timing of transplantation. In relapsed, chemosensitive diffuse large B-cell lymphoma (DLBCL), although post auto-HCT maintenance rituximab seems to be safe and feasible, it does not provide improved survival outcomes and is not recommended. The preliminary results with anti- programmed death -1 (PD-1) antibody therapy as post auto-HCT maintenance in DLBCL is promising but requires randomized validation. Similarly in follicular lymphoma, maintenance therapies including rituximab following auto-HCT should be considered investigational and offered only on a clinical trial. Rituximab maintenance results in improved progression-free survival but has not yet shown to improve overall survival in mantle cell lymphoma (MCL), but given the poor prognosis with post auto-HCT failure in MCL, maintenance rituximab can be considered on a case-by-case basis. Ongoing trials evaluating the efficacy of post auto-HCT maintenance with novel compounds (*e.g.*, immunomodulators, PD-1 inhibitors, proteasome inhibitors and bruton's tyrosine kinase inhibitors) will likely change the practice landscape in the near future for B cell non-Hodgkin lymphomas patients following HDT and auto-HCT.

Key words: Mantle cell lymphoma; Diffuse large B cell lymphoma; Follicular lymphoma; Autologous hematopoietic cell transplantation

Core tip: Prevention of disease-relapse is an unmet medical need in B-cell non-Hodgkin lymphomas (NHL) undergoing autologous hematopoietic cell transplantation (auto-HCT). In this review, are summarized potentially paradigm changing advances in post auto-HCT, maintenance strategies in B-cell NHL.

Epperla N, Fenske TS, Hari PN, Hamadani M. Recent advances in post autologous transplantation maintenance therapies in B-cell non-Hodgkin lymphomas. *World J Transplant* 2015; 5(3): 81-88 Available from: URL: http://www.wjgnet.com/2220-3230/full/v5/i3/81.htm DOI: http://dx.doi.org/10.5500/wjt.v5.i3.81

INTRODUCTION

Hodgkin and non-Hodgkin lymphomas (NHL) collectively constitute the second most common indication for high dose therapy (HDT) and autologous hematopoietic cell transplantation (auto-HCT)[1]. In chemotherapy responsive relapsed lymphoid malignancies auto-HCT can provide long-term disease control, while avoiding the immunologic complications and delayed immune reconstitution associated with allogeneic HCT.

The curative potential of auto-HCT or the expected duration of disease control in lymphoid malignancies varies depending on the histological subtype, number of prior therapy lines and depth of remission prior to HDT. The role of auto-HCT as a potentially curative option in relapsed, chemosensitive diffuse large B-cell lymphoma (DLBCL) is well-defined. The PARMA trial[2] established that salvage chemotherapy and auto-HCT provided a significantly better event-free survival (EFS) and overall survival (OS) in subjects randomized to the HDT arm. Several registry based[3-6] and prospective studies in the rituximab-era[7] have reproduced these results. In contrast, auto-HCT when applied upfront for mantle cell lymphoma (MCL)[8], or for relapsed, chemosensitive patients with follicular lymphoma (FL) is generally not considered a curative modality.

Regardless of the underlying histology or remission status at transplantation, disease relapse or progression remains the number one cause of post auto-HCT therapy failure and mortality. Prevention of disease relapse following auto-HCT in lymphoid malignancies therefore remains an unmet medical need. Disease relapse following auto-HCT occurs *via* two possible mechanisms. Most patients relapse likely due to the proliferation of a resistant clone of lymphoma cells (or stem cells) surviving the HDT. A minority may experience relapse due to re-infusion of an autograft contaminated by lymphoma cells[9]. In order to circumvent the problem of autograft contamination by lymphoma cells, several studies have examined the role of *ex vivo* purging (by monoclonal antibodies, $CD34^+$ cell selection, *etc.*)[10,11] and *in vivo* purging (*e.g.,* rituximab with mobilization)[12,13] of autologous stem cell products. However, randomized data do not demonstrate improved outcomes with purged auto-HCT[14]. Similarly intensifying HDT with radioimmunotherapy based conditioning regimens[15] have likewise not demonstrated improved HCT outcomes. A handful of studies have looked at tandem auto-HCT following by reduced-intensity allogeneic HCT in lymphoid malignancies[16,17]. However no randomized data are available to support the use of this approach. Moreover advanced age, comorbidities and suitable donor availability makes such a tandem HCT approach theoretically applicable to only a small subset of lymphoma patients.

Over the last decade several studies have shown improved outcomes with maintenance immunotherapies applied after conventional chemoimmunotherapies in patients with lymphoid malignancies[18-20]. Owing to the excellent safety profile of maintenance immunotherapies in the non-transplant setting, this modality has now been investigated post auto-HCT in lymphoid malignancies. In this article we review the role of post auto-HCT maintenance therapies in B cell NHL, along with overview of novel agents that likely will serve as future maintenance strategies in the post auto-HCT setting.

DIFFUSE LARGE B CELL LYMPHOMA

Of DLBCL patients who relapse after auto-HCT, a vast majority relapse early post-transplant. In a recent Center for International Blood and Marrow Transplant Research (CIBMTR) study[5], nearly three quarter of relapses in DLBCL were seen within the first 9 mo following autoHCT. A landmark analysis of DLBCL patients surviving the first 9 mo post-transplant without relapse/progression, showed a 5-year progression-free survival (PFS) probability of > 80%, suggesting that an effective strategy to prevent early DLBCL relapses post auto-HCT would theoretically translate into significant improvements in patient outcomes.

Studies evaluating the role of maintenance therapies in DLBCL are summarized in Table 1[21-26]. A small case series by Lim *et al*[21] (n = 15) provided preliminary evidence for maintenance in DLBCL post HCT. In this study post auto-HCT rituximab maintenance in high risk NHL for 2 years (once every 3 mo) provided a relapse-free survival of 100% and OS of 80% at 5.5 years (Table 1). Subsequently, in a small prospective study (n = 12), *in vivo* graft purging and post auto-HCT maintenance with rituximab in high risk DLBCL resulted in 3 year PFS of 83% and OS of 100%[22].

These studies paved way for a large prospective randomized study, in which high-risk DLBCL (n = 269) patients after undergoing an upfront autoHCT consolidation in first remission, were randomized to a brief rituximab course (four weekly doses) *vs* observation. In patients who achieved a complete remission (CR) following HDT, this brief maintenance rituximab exposure provided statistically significant superior EFS (Table 1)[24]. Since all DLBCL patients underwent an auto-HCT

Table 1 Studies evaluating the role of antibody based maintenance therapy post autologous hematopoietic cell transplantation in diffuse large B cell lymphoma

Ref.	Study design	Maintenance schedule	*n*	% CS at HCT	PFS/EFS (%)	OS (%)	Comments
Lim *et al*[21]	Retrospective	Rituximab 375 mg/m^2 (q 3 mo for a total of 8 doses)	15	100	-	80 (5.5 yr)	Relapse free survival 100% (5.5 yr)
Zhang *et al*[22]	Single arm prospective	Rituximab 375 mg/m^2 (q 3 mo for 2 yr)	12	100	83 (3 yr)	100 (3 yr)	Prolonged hypogammaglobinemia in 2 patients
Tsirigotis *et al*[23]	Retrospective	Rituximab 375 mg/m^2 (80% q wk and 20% q mo)	19	79	NR	NR	Compared to controls, maintenance improves PFS and OS
Haioun *et al*[24]	Randomized prospective	Rituximab 375 mg/m^2 (weekly for 4 doses)	269 R = 139, O = 130	84.5	80 (R) *vs* 71 (O) (4 yr)	-	Patients underwent autoHCT upfront in first remission
Gisselbrecht *et al*[25]	Randomized prospective	Rituximab 375 mg/m^2 (q 8 wk for 1 yr)	242 R = 122, O = 120	100	52 (R) *vs* 56 (O) (4 yr)	61 (R) *vs* 65 (O) (4 yr)	4 yr EFS was 52% for Rituximab arm while 53% for observation arm
Armand *et al*[26]	Prospective phase Ⅱ	Pidilizumab 1.5 mg/kg (q 42 d for 3 cycles)	66	91	72 (16 mo)	85 (16 mo)	ORR was 51% (CR of 34%) in pts with measurable disease after autoHCT

CS: Chemo-sensitive; PFS: Progression free survival; OS: Overall survival; NR: Not reached; R: Rituximab arm; O: Observation arm; EFS: Event free survival; ORR: Overall response rate; CR: Complete remission; HCT: Hematopoietic cell transplantation.

in first remission in this trial (a scenario that would not be considered standard-of-care today), caution must be exercised in extrapolation of these data to relapsed DLBCL patients undergoing auto-HCT. Of note, quality of life (QOL) assessments in this study showed rapid recovery (as early as day 100) in all the tested QOL subdomains after auto-HCT and rituximab maintenance did not negatively influence the QOL outcomes[27].

The more clinically relevant question of rituximab maintenance in DLBCL patients after failing first line therapies was addressed in the collaborative trial in relapsed aggressive lymphoma (CORAL) study. In this trial (after an initial randomization of patients between two different salvage therapies), a second randomization of relapsed DLBCL patients after auto-HCT to either rituximab maintenance (every 2 mo for 1 year) or observation alone was performed (Table 1). Rituximab maintenance in this study provided no benefit in terms of EFS, PFS or OS. However an unplanned subset analysis suggested a possible benefit of maintenance rituximab in female patients[25]. This finding likely is a reflection of less rapid rituximab clearance in females, which in turn leads to higher blood concentrations of rituximab[28]. This observation could suggest a benefit of rituximab post auto-HCT in female subjects (and possibly in males using higher doses of rituximab), but this hypothesis needs further investigation. In addition to a lack of randomized data supporting using of maintenance rituximab for relapsed DLBCL, uncontrolled data suggest prolonged hypogammaglobulinemia extending beyond 2 years when using this approach in the post auto-HCT setting[21,22].

Advances in our understanding of tumor biology have led to the development of novel targeted therapies in DLBCL. Programmed death 1 (PD-1) is a T cell co-receptor that binds to the ligand B7 to maintain an immunosuppressive tumor microenvironment. PD-L1 is expressed on suppressor immune cells in the tumor microenvironment and in a subset of DLBCL[29-32] where it may alter the composition and function of tumor-infiltrating lymphocytes[33], and therefore represents a valid therapeutic target. Early after auto-HCT, a majority of the circulating leukocytes are natural killer cells, CD45RO+ memory/effector cells and monocytes, which comprise anti PD-1 monoclonal antibody target populations and whose presence has been associated with a favorable prognosis in DLBCL[34-36]. In DLBCL patients, post auto-HCT PD-1 blockade may prevent PD-1 mediated exhaustion of antitumor lymphocytes, leading to eradication of residual disease and improvement in transplant outcomes. In a multicenter phase Ⅱ trial (Table 1) an anti-PD-1 monoclonal antibody, pidilizumab, was administered to patients with relapsed or refractory DBLCL following auto-HCT. The 16-mo PFS was 72% in the overall population and 70% in the subgroup of high-risk patients who had a positive positron emission tomography scan at the end of salvage therapy. Remarkably, 51% of patients with residual disease after transplant responded to the treatment, and 34% of these patients had CR without significant autoimmune toxicity[26]. Although promising, these results have not been confirmed in a prospective randomized trial yet.

Several ongoing trials are looking at maintenance post auto-HCT in DLBCL using immune modulators (NCT01241734; lenalidomide maintenance; phase Ⅰ/Ⅱ), PD-1 inhibitors (NCT02362997; pembrolizumab; phase Ⅱ), proteasome inhibitors (NCT00992446; bortezomib in combination with vorinostat; phase Ⅱ) and Bruton's tyrosine kinase inhibitors[37] (ibrutinib maintenance in activated B-cell type DLBCL in the soon to open BMT-CTN/Alliance phase Ⅲ study).

Table 2 Studies evaluating the role of rituximab maintenance after autologous hematopoietic cell transplantation in mantle cell lymphoma

Ref.	Design	Maintenance	*n*	% CS at HCT	PFS/EFS (%)	OS (%)	Comments
Lim *et al*[46]	Retrospective	Rituximab 375 mg/m^2 (q 3 mo for 2 yr starting day + 100)	8	100	57	67	Delayed immunoglobulin reconstitution was seen in all patients and persisted beyond the rituximab maintenance period
Graf *et al*[47]	Retrospective	Rituximab 375 mg/m^2 (variable dosing schedule but median doses = 8)	157 R = 50, O = 107	Almost all the patients who received MR	HR of 0.33	HR of 0.40	In the landmark analysis at D 100 after auto-HCT 3 yr PFS and OS were statistically better in the MR compared to the no MR group
Dietrich *et al*[48]	Retrospective	Rituximab 375 mg/m^2 (every 3 mo for 2 yr)	72 R = 22, O = 50		90 (R) *vs* 65 (O)	90 (R) *vs* 84 (O)	Patients in both the arms were well matched. The median observation time was 56 mo
Gouill *et al*[49]	Prospective phase Ⅲ	Rituximab 375 mg/m^2 Ⅳ (every 2 mo for 3 yr)	238 R = 119, O = 119	81.4	93.2 (R) *vs* 81.5 (O) (2 yr)	93.4 (R) *vs* 93.9 (O) (2 yr)	All patients received 4 courses of R-DHAP followed by auto-HCT. The conditioning regimen of auto-HCT was R-BEAM (R=500 mg/m^2)

CS: Chemo-sensitive; PFS: Progression free survival; EFS: Event free survival; OS: Overall survival; MR: Maintenance rituximab; HR: Hazard ratio; R: Rituximab arm; O: Observation arm; R-DHAP: Rituximab, dexamethasone, cytarabine and cisplatin; R-BEAM: Rituximab, carmustine, etoposide, cytarabine and melphalan; HCT: Hematopoietic cell transplantation.

Bottom-line

Although rituximab seems to be a feasible and safe option post auto-HCT, it does not provide improved disease control or survival outcomes and is not recommended in this setting. The preliminary results with PD-1 antibody as a post auto-HCT maintenance therapy in DLBCL are promising but require validation in a randomized setting.

FOLLICULAR LYMPHOMA

Registry data from the European Group for Blood and Marrow Transplantation (EBMT)[38] and the CIBMTR show no plateau in relapse rates of FL after auto-HCT[39]. Since maintenance immunotherapies (with rituximab) in FL have shown benefit after both frontline[18] and subsequent chemoimmunotherapies[40,41], the application of rituximab maintenance following auto-HCT would also be a reasonable strategy to potentially prevent relapse.

The EBMT recently reported the efficacy and safety of rituximab, as *in vivo* purging before transplantation and as maintenance treatment immediately after HDT and auto-HCT in patients with relapsed FL, in a randomized prospective trial. In this study, 280 rituximab-naïve patients with relapsed FL were randomly assigned to auto-HCT with or without *in vivo* rituximab purging, followed by a second randomization to rituximab maintenance therapy (once every 2 mo for a total of four infusions) or observation[42]. At a median follow-up of 8.3 years, rituximab maintenance when compared to observation resulted in superior PFS at 10 years (54% *vs* 37%), but did not translate into an improvement in OS (73% *vs* 68%)[42]. In addition, maintenance rituximab was associated with a higher (albeit statistically non-significant) rate of late neutropenia. Considering the fact that this study enrolled rituximab-naïve patients, the lack of a survival benefit in this study is particularly noteworthy. It is plausible that the relatively short maintenance schedule employed in this trial resulted in a lack of survival benefit. Though randomized trials in FL in the non-transplant setting have shown no OS or PFS benefit with rituximab maintenance when using a shorter course (about 8 mo) of maintenance, as used in the EBMT study[43], the Swiss study [Swiss Group for Clinical Cancer Research (SAKK 35/98)] demonstrated superior EFS[44].

While rituximab maintenance post auto-HCT appears unlikely to improve survival of FL patients, the role of other novel approaches as maintenance therapies post auto-HCT in follicular lymphoma warrants further investigation. Ongoing post auto-HCT maintenance clinical trials involving FL patients are evaluating the role of immune modulators (NCT01035463; lenalidomide maintenance; phase Ⅰ/Ⅱ), and proteasome inhibitors (NCT00992446; bortezomib in combination with vorinostat; phase Ⅱ) as maintenance options.

Bottom-line

Maintenance therapies including rituximab following autoHCT should be considered investigational in patients with FL and should only be offered on a clinical trial.

MCL

Maintenance rituximab after induction chemoimmunotherapies has been shown to improve OS in older patients with MCL[20]. In MCL, prevention of relapse or progression after auto-HCT is crucial; since outcome after auto-HCT relapse is dismal with a median survival of only 23 mo[45]. Several retrospective and a few prospective studies have evaluated the potential role of post auto-HCT maintenance rituximab in MCL (Table 2)[46-49].

Dietrich *et al*[48] compared post auto-HCT maintenance rituximab (administered within a prospective phase Ⅱ study of rituximab maintenance in B-cell lymphoma NCT 01933711), to MCL patients getting no maintenance (but transplanted during the same

Table 3 Future directions - drugs that are currently studied in relapsed/refractory aggressive and indolent B cell lymphomas that can potentially be studied in the post autologous hematopoietic cell transplantation setting

Drug	Mechanism of action	Ongoing trials in relapsed/refractory aggressive and indolent B cell lymphomas (not in post auto-HCT setting)
CD-19 antibodies (MEDI-551)	IgG1k antibody-dependent cellular cytotoxicity enhanced anti-CD19 mAb	Phase Ⅰ (NCT00983619) Phase Ⅱ (with ICE/DHAP NCT01453205) Phase Ⅱ (with PD-1 inhibitor NCT02271945)
MPDL3280A	Targets PD-L1 expressed on tumor cells and tumor-infiltrating immune cells	Phase Ⅰ (with Obinutuzumab NCT02220842)
Polatuzumab vedotin	Antibody-drug conjugate that targets CD 79b on the B cell receptor complex	Phase Ⅱ (with Rituximab or Obinutuzumab and Bendamustine NCT02257567)
Obinutuzumab (GA101)	Fully humanized IgG1 mAb that selectivity binds to the extracellular domain of the human CD20 antigen on malignant human B cells	Phase Ⅰ b/ Ⅱ (with lenalidomide NCT01582776) Phase Ⅰ b/ Ⅱ (with lenalidomide NCT01995669)
Veltuzumab	A fully humanized mAb directed against the CD20 antigen.	Phase Ⅰ / Ⅱ (NCT01147393)
ABT-199	Oral selective small molecule inhibitor of the anti-apoptotic protein Bcl-2	Phase Ⅰ (NCT02055820) Phase Ⅰ (with BR NCT01594229) Phase Ⅱ (with BR *vs* BR alone NCT02187861)
Alisertib	Oral selective small molecule inhibitor of the serine/threonine protein kinase Aurora A kinase	Phase Ⅰ (with Romidepsin NCT01897012) Phase Ⅰ (with Vorinostat NCT01567709) Phase Ⅰ (with Bortezomib and Rituximab NCT01695941) Phase Ⅱ (with +/- Rituximab NCT01812005)
SAR245409	Oral small molecule targeting the PI3K and mTOR kinases.	Phase Ⅰ / Ⅱ (NCT01587040)
Belinostat	HDAC inhibitor	Phase Ⅰ (with Carfilzomib NCT02142530) Phase Ⅱ (with Ibritumomab Tiuxetan NCT01686165)

ICE: Ifosfamide, carboplatin and etoposide; DHAP: Dexamethasone, high dose cytarabine, cisplatin; PD-1: Programmed death-1; BR: Bendamustine, rituximab; mAb: Monoclonal antibody; PI3K: Phosphatidylinositol 3 kinase; mTOR: Mammalian target of rapamycin.

time period of aforementioned trial). The study showed that the 2 year PFS was significantly better in the maintenance rituximab compared to no maintenance rituximab cohort (90% and 65% respectively $P = 0.014$) with no difference in OS between the two arms (90% in maintenance rituximab and 84% in no maintenance rituximab) (Table 2). However, following a multivariate adjustment for other factors maintenance rituximab was strongly associated with both PFS and OS[48].

The only randomized phase Ⅲ trial to study maintenance therapy in post auto-HCT setting in MCL was conducted by the LYSA, GOELAMS (Groupe Ouest Est d'Etude des Leucémies et Autres Maladies du Sang) and GELA (Groupe d'Etude des Lymphomes de l'Adulte). Patients who achieved a CR or partial remission to auto-HCT ($n = 238$) were randomized to maintenance rituximab ($n = 119$) (375 mg/m^2, IV every 2 mo for 3 years) or wait and watch (WW) ($n = 119$) arms. The 2 year EFS and PFS were statistically different between the two arms ($P = 0.015$ for both) favoring the maintenance rituximab (93.2% in the maintenance rituximab arm *vs* 81.5% in the WW arm), however there was no difference in OS (93.4% in the maintenance rituximab arm *vs* 93.9% in the WW arm) (Table 2)[49]. Final data with mature follow up and complete toxicity assessment is not yet reported.

Among lymphoid malignancies, the therapeutic landscape of MCL is rapidly changing with several new agents approved for therapy in relapsed/refractory setting in the last 2-3 years. Lenalidomide has shown significant activity in relapsed/refractory MCL leading to its approval as a single agent in this patient group[50]. Fondazione Italiana Linfomi ongoing randomized phase Ⅲ study is evaluating the role of lenalidomide maintenance after upfront auto-HCT consolidation in MCL (NCT02354313). Ibrutinib, another agent with known activity in relapsed MCL[51] is a potential candidate for post auto-HCT maintenance. A single arm prospective trial is administrating ibrutinib as maintenance therapy after intensive induction programs (with or without auto-HCT) (NCT02242097). Minimal residual monitoring (MRD) monitoring with polymerase chain reaction (PCR) for immunoglobulin heavy chain (IgH) and/or bcl-1 rearrangement was employed in the MCL-2 trial[52]. Pre-emptive treatment with rituximab achieved a second molecular remission in 92% of the patients ($n = 26$) experiencing molecular relapse (PCR+ for IgH rearrangement) post auto-HCT. After pre-emptive treatment median clinical and molecular relapse free survivals were 3.7 and 1.5 years respectively. Though strictly speaking pre-emptive therapy is not post-transplant maintenance, it is akin to the post auto-HCT maintenance therapy but needs further investigation.

Bottom-line

Considering the poor prognosis to post auto-HCT failures in MCL, rituximab maintenance should be evaluated on a case-by-case basis (*e.g.,* patients who would not be fit for a subsequent allogeneic transplant). In addition, rationale application of novel maintenance therapies using MRD monitoring represents a promising investigational approach for MCL patients after auto-HCT.

ON THE HORIZON

Moving forward, to further improve outcomes for NHL patients undergoing auto-HCT, efforts need to be focused on evaluating novel consolidation or maintenance strategies, possibly with agents not used in induction chemoimmunotherapies. Table 3 summarizes the novel agents that are currently being studied in relapsed/refractory aggressive and indolent B cell NHL. Consolidation and/or maintenance with monoclonal antibodies [to cite a few - anti CD 79b (Polatuzumab Vedotin), anti CD19 (MEDI 551) and anti CD20 (Obinutuzumab and Veltuzumab)], HDAC inhibitors (Belinostat), PDL-1 inhibitors (MPDL3280A), Bcl-2 inhibitors (ABT-199), Aurora A kinase inhibitors (Alisertib) and mTOR/PI3K inhibitors (SAR245409) in the post auto-HCT setting seems to be a potential area of further investigation.

REFERENCES

1 **Pasquini M**, Wang Z, Horowitz MM, Gale RP. 2013 report from the Center for International Blood and Marrow Transplant Research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. *Clin Transpl* 2013: 187-197 [PMID: 25095508]

2 **Philip T**, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D, Sonneveld P, Gisselbrecht C, Cahn JY, Harousseau JL. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med* 1995; **333**: 1540-1545 [PMID: 7477169 DOI: 10.1056/NEJM199512073332305]

3 **Vose JM**, Zhang MJ, Rowlings PA, Lazarus HM, Bolwell BJ, Freytes CO, Pavlovsky S, Keating A, Yanes B, van Besien K, Armitage JO, Horowitz MM. Autologous transplantation for diffuse aggressive non-Hodgkin's lymphoma in patients never achieving remission: a report from the Autologous Blood and Marrow Transplant Registry. *J Clin Oncol* 2001; **19**: 406-413 [PMID: 11208832]

4 **Mounier N**, Canals C, Gisselbrecht C, Cornelissen J, Foa R, Conde E, Maertens J, Attal M, Rambaldi A, Crawley C, Luan JJ, Brune M, Wittnebel S, Cook G, van Imhoff GW, Pfreundschuh M, Sureda A. High-dose therapy and autologous stem cell transplantation in first relapse for diffuse large B cell lymphoma in the rituximab era: an analysis based on data from the European Blood and Marrow Transplantation Registry. *Biol Blood Marrow Transplant* 2012; **18**: 788-793 [PMID: 22005647 DOI: 10.1016/j.bbmt.2011.10.010]

5 **Hamadani M**, Hari PN, Zhang Y, Carreras J, Akpek G, Aljurf MD, Ayala E, Bachanova V, Chen AI, Chen YB, Costa LJ, Fenske TS, Freytes CO, Ganguly S, Hertzberg MS, Holmberg LA, Inwards DJ, Kamble RT, Kanfer EJ, Lazarus HM, Marks DI, Nishihori T, Olsson R, Reddy NM, Rizzieri DA, Savani BN, Solh M, Vose JM, Wirk B, Maloney DG, Smith SM, Montoto S, Saber W, Alpdogan O, Cashen A, Dandoy C, Finke R, Gale R, Gibson J, Hsu JW, Janakiraman N, Laughlin MJ, Lill M, Cairo MS, Munker R, Rowlings PA, Schouten HC, Shea TC, Stiff PJ, Waller EK. Early failure of frontline rituximab-containing chemo-immunotherapy in diffuse large B cell lymphoma does not predict futility of autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2014; **20**: 1729-1736 [PMID: 25008330 DOI: 10.1016/j.bbmt.2014.06.036]

6 **Fenske TS**, Hari PN, Carreras J, Zhang MJ, Kamble RT, Bolwell BJ, Cairo MS, Champlin RE, Chen YB, Freytes CO, Gale RP, Hale GA, Ilhan O, Khoury HJ, Lister J, Maharaj D, Marks DI, Munker R, Pecora AL, Rowlings PA, Shea TC, Stiff P, Wiernik PH, Winter JN, Rizzo JD, van Besien K, Lazarus HM, Vose JM. Impact of pre-transplant rituximab on survival after autologous hematopoietic stem cell transplantation for diffuse large B cell lymphoma. *Biol Blood Marrow Transplant* 2009; **15**: 1455-1464 [PMID: 19822306 DOI: 10.1016/j.bbmt.2009.07.017]

7 **Gisselbrecht C**, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, Bosly A, Ketterer N, Shpilberg O, Hagberg H, Ma D, Brière J, Moskowitz CH, Schmitz N. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol* 2010; **28**: 4184-4190 [PMID: 20660832 DOI: 10.1200/JCO.2010.28.1618]

8 **Fenske TS**, Zhang MJ, Carreras J, Ayala E, Burns LJ, Cashen A, Costa LJ, Freytes CO, Gale RP, Hamadani M, Holmberg LA, Inwards DJ, Lazarus HM, Maziarz RT, Munker R, Perales MA, Rizzieri DA, Schouten HC, Smith SM, Waller EK, Wirk BM, Laport GG, Maloney DG, Montoto S, Hari PN. Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-sensitive mantle-cell lymphoma: analysis of transplantation timing and modality. *J Clin Oncol* 2014; **32**: 273-281 [PMID: 24344210 DOI: 10.1200/JCO.2013.49.2454]

9 **Hamadani M**. Reappraising the role of autologous transplantation for indolent B-cell lymphomas in the chemoimmunotherapy era: is it still relevant? *Bone Marrow Transplant* 2013; **48**: 1013-1021 [PMID: 23000653 DOI: 10.1038/bmt.2012.182]

10 **Freedman AS**, Neuberg D, Mauch P, Soiffer RJ, Anderson KC, Fisher DC, Schlossman R, Alyea EP, Takvorian T, Jallow H, Kuhlman C, Ritz J, Nadler LM, Gribben JG. Long-term follow-up of autologous bone marrow transplantation in patients with relapsed follicular lymphoma. *Blood* 1999; **94**: 3325-3333 [PMID: 10552941]

11 **Tarella C**, Corradini P, Astolfi M, Bondesan P, Caracciolo D, Cherasco C, Ladetto M, Giaretta F, Ricca I, Vitolo U, Pileri A, Ferrero D. Negative immunomagnetic ex vivo purging combined with high-dose chemotherapy with peripheral blood progenitor cell autograft in follicular lymphoma patients: evidence for long-term clinical and molecular remissions. *Leukemia* 1999; **13**: 1456-1462 [PMID: 10482999 DOI: 10.1038/sj.leu.2401488]

12 **Tarella C**, Zanni M, Magni M, Benedetti F, Patti C, Barbui T, Pileri A, Boccadoro M, Ciceri F, Gallamini A, Cortelazzo S, Majolino I, Mirto S, Corradini P, Passera R, Pizzolo G, Gianni AM, Rambaldi A. Rituximab improves the efficacy of high-dose chemotherapy with autograft for high-risk follicular and diffuse large B-cell lymphoma: a multicenter Gruppo Italiano Terapie Innnovative nei linfomi survey. *J Clin Oncol* 2008; **26**: 3166-3175 [PMID: 18490650 DOI: 10.1200/JCO.2007.14.4204]

13 **Arcaini L**, Montanari F, Alessandrino EP, Tucci A, Brusamolino E, Gargantini L, Cairoli R, Bernasconi P, Passamonti F, Bonfichi M, Zoli V, Bottelli C, Calatroni S, Troletti D, Merli M, Pascutto C, Majolino I, Rossi G, Morra E, Lazzarino M. Immunochemotherapy with in vivo purging and autotransplant induces long clinical and molecular remission in advanced relapsed and refractory follicular lymphoma. *Ann Oncol* 2008; **19**: 1331-1335 [PMID: 18344536 DOI: 10.1093/annonc/mdn044]

14 **Schouten HC**, Qian W, Kvaloy S, Porcellini A, Hagberg H, Johnsen HE, Doorduijn JK, Sydes MR, Kvalheim G. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. *J Clin Oncol* 2003; **21**: 3918-3927 [PMID: 14517188 DOI: 10.1200/JCO.2003.10.023]

15 **Vose JM**, Carter S, Burns LJ, Ayala E, Press OW, Moskowitz CH, Stadtmauer EA, Mineshi S, Ambinder R, Fenske T, Horowitz M, Fisher R, Tomblyn M. Phase III randomized study of rituximab/carmustine, etoposide, cytarabine, and melphalan (BEAM) compared with iodine-131 tositumomab/BEAM with autologous hematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: results from the BMT CTN 0401 trial. *J Clin Oncol* 2013; **31**: 1662-1668 [PMID: 23478060 DOI: 10.1200/JCO.2012.45.9453]

16 **Cohen S**, Kiss T, Lachance S, Roy DC, Sauvageau G, Busque L, Ahmad I, Roy J. Tandem autologous-allogeneic nonmyeloablative sibling transplantation in relapsed follicular lymphoma leads to impressive progression-free survival with minimal toxicity. *Biol Blood Marrow Transplant* 2012; **18**: 951-957 [PMID: 22155507

DOI: 10.1016/j.bbmt.2011.11.028]

17 **Satwani P**, Jin Z, Martin PL, Bhatia M, Garvin JH, George D, Chaudhury S, Talano J, Morris E, Harrison L, Sosna J, Peterson M, Militano O, Foley S, Kurtzberg J, Cairo MS. Sequential myeloablative autologous stem cell transplantation and reduced intensity allogeneic hematopoietic cell transplantation is safe and feasible in children, adolescents and young adults with poor-risk refractory or recurrent Hodgkin and non-Hodgkin lymphoma. *Leukemia* 2015; **29**: 448-455 [PMID: 24938649 DOI: 10.1038/leu.2014.194]

18 **Salles G**, Seymour JF, Offner F, López-Guillermo A, Belada D, Xerri L, Feugier P, Bouabdallah R, Catalano JV, Brice P, Caballero D, Haioun C, Pedersen LM, Delmer A, Simpson D, Leppa S, Soubeyran P, Hagenbeek A, Casasnovas O, Intragumtornchai T, Fermé C, da Silva MG, Sebban C, Lister A, Estell JA, Milone G, Sonet A, Mendila M, Coiffier B, Tilly H. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. *Lancet* 2011; **377**: 42-51 [PMID: 21176949 DOI: 10.1016/S0140-6736(10)62175-7]

19 **Kahl BS**, Hong F, Williams ME, Gascoyne RD, Wagner LI, Krauss JC, Habermann TM, Swinnen LJ, Schuster SJ, Peterson CG, Sborov MD, Martin SE, Weiss M, Ehmann WC, Horning SJ. Rituximab extended schedule or re-treatment trial for low-tumor burden follicular lymphoma: eastern cooperative oncology group protocol e4402. *J Clin Oncol* 2014; **32**: 3096-3102 [PMID: 25154829 DOI: 10.1200/JCO.2014.56.5853]

20 **Kluin-Nelemans HC**, Hoster E, Hermine O, Walewski J, Trneny M, Geisler CH, Stilgenbauer S, Thieblemont C, Vehling-Kaiser U, Doorduijn JK, Coiffier B, Forstpointner R, Tilly H, Kanz L, Feugier P, Szymczyk M, Hallek M, Kremers S, Lepeu G, Sanhes L, Zijlstra JM, Bouabdallah R, Lugtenburg PJ, Macro M, Pfreundschuh M, Procházka V, Di Raimondo F, Ribrag V, Uppenkamp M, André M, Klapper W, Hiddemann W, Unterhalt M, Dreyling MH. Treatment of older patients with mantle-cell lymphoma. *N Engl J Med* 2012; **367**: 520-531 [PMID: 22873532 DOI: 10.1056/NEJMoa1200920]

21 **Lim SH**, Esler WV, Zhang Y, Zhang J, Periman PO, Burris C, Townsend M. B-cell depletion for 2 years after autologous stem cell transplant for NHL induces prolonged hypogammaglobulinemia beyond the rituximab maintenance period. *Leuk Lymphoma* 2008; **49**: 152-153 [PMID: 18203024 DOI: 10.1080/10428190701742506]

22 **Zhang W,** Jiao L, Zhou DB, Shen T. Rituximab purging and maintenance therapy combined with autologous stem cell transplantation in patients with diffuse large B-cell lymphoma. *Oncol Lett* 2010; **1**: 733-738 [PMID: 22966371]

23 **Tsirigotis P**, Dray L, Resnick IB, Ackerstein A, Gesundheit B, Elad S, Or R, Shapira MY. Post-autologous stem cell transplantation administration of rituximab improves the outcome of patients with aggressive B cell non-Hodgkin's lymphoma. *Ann Hematol* 2010; **89**: 263-272 [PMID: 19693502 DOI: 10.1007/s00277-009-0808-5]

24 **Haioun C**, Mounier N, Emile JF, Ranta D, Coiffier B, Tilly H, Récher C, Fermé C, Gabarre J, Herbrecht R, Morchhauser F, Gisselbrecht C. Rituximab versus observation after high-dose consolidative first-line chemotherapy with autologous stem-cell transplantation in patients with poor-risk diffuse large B-cell lymphoma. *Ann Oncol* 2009; **20**: 1985-1992 [PMID: 19567453 DOI: 10.1093/annonc/mdp237]

25 **Gisselbrecht C**, Schmitz N, Mounier N, Singh Gill D, Linch DC, Trneny M, Bosly A, Milpied NJ, Radford J, Ketterer N, Shpilberg O, Dührsen U, Hagberg H, Ma DD, Viardot A, Lowenthal R, Brière J, Salles G, Moskowitz CH, Glass B. Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20(+) diffuse large B-cell lymphoma: final analysis of the collaborative trial in relapsed aggressive lymphoma. *J Clin Oncol* 2012; **30**: 4462-4469 [PMID: 23091101 DOI: 10.1200/JCO.2012.41.9416]

26 **Armand P**, Nagler A, Weller EA, Devine SM, Avigan DE, Chen YB, Kaminski MS, Holland HK, Winter JN, Mason JR, Fay JW, Rizzieri DA, Hosing CM, Ball ED, Uberti JP, Lazarus HM, Mapara MY, Gregory SA, Timmerman JM, Andorsky D, Or R, Waller EK, Rotem-Yehudar R, Gordon LI. Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. *J Clin Oncol* 2013; **31**: 4199-4206 [PMID: 24127452 DOI: 10.1200/JCO.2012.48.3685]

27 **Heutte N**, Haioun C, Feugier P, Coiffier B, Tilly H, Ferme C, Gabarre J, Morschhauser F, Gisselbrecht C, Mounier N. Quality of life in 269 patients with poor-risk diffuse large B-cell lymphoma treated with rituximab versus observation after autologous stem cell transplant. *Leuk Lymphoma* 2011; **52**: 1239-1248 [PMID: 21463114 DOI: 10.3109/10428194.2011.566951]

28 **Pfreundschuh M**, Poeschel V, Zeynalova S, Hänel M, Held G, Schmitz N, Viardot A, Dreyling MH, Hallek M, Mueller C, Wiesen MH, Witzens-Harig M, Truemper L, Keller U, Rixecker T, Zwick C, Murawski N. Optimization of rituximab for the treatment of diffuse large B-cell lymphoma (II): extended rituximab exposure time in the SMARTE-R-CHOP-14 trial of the german high-grade non-Hodgkin lymphoma study group. *J Clin Oncol* 2014; **32**: 4127-4133 [PMID: 25403207 DOI: 10.1200/JCO.2013.54.6861]

29 **Andorsky DJ,** Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. *Clin Cancer Res* 2011; **17**: 4232-4244. [PMID: 21540239 DOI: 10.1158/1078-0432.CCR-10-2660]

30 **Li Y**, Wang J, Li C, Ke XY. Contribution of PD-L1 to oncogenesis of lymphoma and its RNAi-based targeting therapy. *Leuk Lymphoma* 2012; **53**: 2015-2023 [PMID: 22462616 DOI: 10.3109/10428194.2012.673228]

31 **Green MR**, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, Chapuy B, Takeyama K, Neuberg D, Golub TR, Kutok JL, Shipp MA. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood* 2010; **116**: 3268-3277 [PMID: 20628145 DOI: 10.1182/blood-2010-05-282780]

32 **Rosenwald A**, Wright G, Leroy K, Yu X, Gaulard P, Gascoyne RD, Chan WC, Zhao T, Haioun C, Greiner TC, Weisenburger DD, Lynch JC, Vose J, Armitage JO, Smeland EB, Kvaloy S, Holte H, Delabie J, Campo E, Montserrat E, Lopez-Guillermo A, Ott G, Muller-Hermelink HK, Connors JM, Braziel R, Grogan TM, Fisher RI, Miller TP, LeBlanc M, Chiorazzi M, Zhao H, Yang L, Powell J, Wilson WH, Jaffe ES, Simon R, Klausner RD, Staudt LM. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med* 2003; **198**: 851-862 [PMID: 12975453 DOI: 10.1084/jem.20031074]

33 **Warlick ED**, Tomblyn M, Cao Q, Defor T, Blazar BR, Macmillan M, Verneris M, Wagner J, Dusenbery K, Aurora M, Bachanova V, Brunstein C, Burns L, Cooley S, Kaufman D, Majhail NS, McClune B, McGlave P, Miller J, Oran B, Slungaard A, Vercellotti G, Weisdorf DJ. Reduced-intensity conditioning followed by related allografts in hematologic malignancies: long-term outcomes most successful in indolent and aggressive non-Hodgkin lymphomas. *Biol Blood Marrow Transplant* 2011; **17**: 1025-1032 [PMID: 21047561 DOI: 10.1016/j.bbmt.2010.10.030]

34 **Ansell SM**, Stenson M, Habermann TM, Jelinek DF, Witzig TE. Cd4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. *J Clin Oncol* 2001; **19**: 720-726 [PMID: 11157023]

35 **Guillaume T**, Rubinstein DB, Symann M. Immune reconstitution and immunotherapy after autologous hematopoietic stem cell transplantation. *Blood* 1998; **92**: 1471-1490 [PMID: 9716573]

36 **Porrata LF**, Litzow MR, Markovic SN. Immune reconstitution after autologous hematopoietic stem cell transplantation. *Mayo Clin Proc* 2001; **76**: 407-412 [PMID: 11322356 DOI: 10.1016/S0025-6196(11)62388-4]

37 **Mathews Griner LA**, Guha R, Shinn P, Young RM, Keller JM, Liu D, Goldlust IS, Yasgar A, McKnight C, Boxer MB, Duveau

DY, Jiang JK, Michael S, Mierzwa T, Huang W, Walsh MJ, Mott BT, Patel P, Leister W, Maloney DJ, Leclair CA, Rai G, Jadhav A, Peyser BD, Austin CP, Martin SE, Simeonov A, Ferrer M, Staudt LM, Thomas CJ. High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci USA* 2014; **111**: 2349-2354 [PMID: 24469833 DOI: 10.1073/pnas.1311846111]

38 **Montoto S**, Canals C, Rohatiner AZ, Taghipour G, Sureda A, Schmitz N, Gisselbrecht C, Fouillard L, Milpied N, Haioun C, Slavin S, Conde E, Fruchart C, Ferrant A, Leblond V, Tilly H, Lister TA, Goldstone AH. Long-term follow-up of high-dose treatment with autologous haematopoietic progenitor cell support in 693 patients with follicular lymphoma: an EBMT registry study. *Leukemia* 2007; **21**: 2324-2331 [PMID: 17637813 DOI: 10.1038/sj.leu.2404850]

39 **van Besien K**, Loberiza FR, Bajorunaite R, Armitage JO, Bashey A, Burns LJ, Freytes CO, Gibson J, Horowitz MM, Inwards DJ, Marks DI, Martino R, Maziarz RT, Molina A, Pavlovsky S, Pecora AL, Schouten HC, Shea TC, Lazarus HM, Rizzo JD, Vose JM. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. *Blood* 2003; **102**: 3521-3529 [PMID: 12893748 DOI: 10.1182/blood-2003-04-1205]

40 **van Oers MH**, Klasa R, Marcus RE, Wolf M, Kimby E, Gascoyne RD, Jack A, Van't Veer M, Vranovsky A, Holte H, van Glabbeke M, Teodorovic I, Rozewicz C, Hagenbeek A. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood* 2006; **108**: 3295-3301 [PMID: 16873669 DOI: 10.1182/blood-2006-05-021113]

41 **van Oers MH**, Van Glabbeke M, Giurgea L, Klasa R, Marcus RE, Wolf M, Kimby E, van t Veer M, Vranovsky A, Holte H, Hagenbeek A. Rituximab maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: long-term outcome of the EORTC 20981 phase III randomized intergroup study. *J Clin Oncol* 2010; **28**: 2853-2858 [PMID: 20439641 DOI: 10.1200/JCO.2009.26.5827]

42 **Pettengell R**, Schmitz N, Gisselbrecht C, Smith G, Patton WN, Metzner B, Caballero D, Tilly H, Walewski JA, Bence-Bruckler I, To B, Geisler CH, Schots R, Kimby E, Taverna CJ, Kozák T, Dreger P, Uddin R, Ruiz de Elvira C, Goldstone AH. Rituximab purging and/or maintenance in patients undergoing autologous transplantation for relapsed follicular lymphoma: a prospective randomized trial from the lymphoma working party of the European group for blood and marrow transplantation. *J Clin Oncol* 2013; **31**: 1624-1630 [PMID: 23547078 DOI: 10.1200/JCO.2012.47.1862]

43 **Vitolo U**, Ladetto M, Boccomini C, Baldini L, De Angelis F, Tucci A, Botto B, Chiappella A, Chiarenza A, Pinto A, De Renzo A, Zaja F, Castellino C, Bari A, Alvarez De Celis I, Evangelista A, Parvis G, Gamba E, Lobetti-Bodoni C, Ciccone G, Rossi G. Rituximab maintenance compared with observation after brief first-line R-FND chemoimmunotherapy with rituximab consolidation in patients age older than 60 years with advanced follicular lymphoma: a phase III randomized study by the Fondazione Italiana Linfomi. *J Clin Oncol* 2013; **31**: 3351-3359 [PMID: 23960180 DOI: 10.1200/JCO.2012.44.8290]

44 **Martinelli G**, Schmitz SF, Utiger U, Cerny T, Hess U, Bassi S, Okkinga E, Stupp R, Stahel R, Heizmann M, Vorobiof D, Lohri A, Dietrich PY, Zucca E, Ghielmini M. Long-term follow-up of patients with follicular lymphoma receiving single-agent rituximab at two different schedules in trial SAKK 35/98. *J Clin Oncol* 2010; **28**: 4480-4484 [PMID: 20697092 DOI: 10.1200/JCO.2010.28.4786]

45 **Dietrich S**, Tielesch B, Rieger M, Nickelsen M, Pott C, Witzens-Harig M, Kneba M, Schmitz N, Ho AD, Dreger P. Patterns and outcome of relapse after autologous stem cell transplantation for mantle cell lymphoma. *Cancer* 2011; **117**: 1901-1910 [PMID: 21509767 DOI: 10.1002/cncr.25756]

46 **Lim SH**, Esler WV, Periman PO, Beggs D, Zhang Y, Townsend M. R-CHOP followed by consolidative autologous stem cell transplant and low dose rituxan maintenance therapy for advanced mantle cell lymphoma. *Br J Haematol* 2008; **142**: 482-484 [PMID: 18510683 DOI: 10.1111/j.1365-2141.2008.07210.x]

47 **Graf SA,** Stevenson PA, Holmberg LA, Till BG, Press OW, Chauncey TR, Smith SD, Philip M, Orozco JJ, Shustov AR, Green DJ, Libby EN, Bensinger WI, Pagel JM, Maloney DG, Zhou Y, Cassaday RD, Gopal AK. Rituximab maintenance therapy after autologous stem cell transplantation improves survival of patients with mantle cell lymphoma. 2014 ASH Annual Meeting;San Francisco, CA, December 6-9, 2014

48 **Dietrich S**, Weidle J, Rieger M, Meissner J, Radujkovic A, Ho AD, Dreger P, Witzens-Harig M. Rituximab maintenance therapy after autologous stem cell transplantation prolongs progression-free survival in patients with mantle cell lymphoma. *Leukemia* 2014; **28**: 708-709 [PMID: 24217198 DOI: 10.1038/leu.2013.332]

49 **Gouill SL,** Thieblemont C, Oberic L, Bouabdallah K, Gyan E, Damaj G, Ribrag V, Bologna S, Gressin R, Casasnovas O, Haioun C, Solal-Celigny P, Maisonneuve H, Neste EVD, Moreau A, Bene MC, Salles G, Tilly H, Lamy T, Hermine O. Rituximab Maintenance Versus Wait and Watch after Four Courses of R-DHAP Followed by Autologous Stem Cell transplantation in Previously Untreated Young Patients with Mantle Cell Lymphoma: First Interim Analysis of the Phase III Prospective Lyma Trial, a Lysa Study. Presented at: 2014 ASH Annual Meeting; San Francisco, CA, December 6-9, 2014

50 **Goy A**, Sinha R, Williams ME, Kalayoglu Besisik S, Drach J, Ramchandren R, Zhang L, Cicero S, Fu T, Witzig TE. Single-agent lenalidomide in patients with mantle-cell lymphoma who relapsed or progressed after or were refractory to bortezomib: phase II MCL-001 (EMERGE) study. *J Clin Oncol* 2013; **31**: 3688-3695 [PMID: 24002500 DOI: 10.1200/JCO.2013.49.2835]

51 **Wang ML**, Rule S, Martin P, Goy A, Auer R, Kahl BS, Jurczak W, Advani RH, Romaguera JE, Williams ME, Barrientos JC, Chmielowska E, Radford J, Stilgenbauer S, Dreyling M, Jedrzejczak WW, Johnson P, Spurgeon SE, Li L, Zhang L, Newberry K, Ou Z, Cheng N, Fang B, McGreivy J, Clow F, Buggy JJ, Chang BY, Beaupre DM, Kunkel LA, Blum KA. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med* 2013; **369**: 507-516 [PMID: 23782157 DOI: 10.1056/NEJMoa1306220]

52 **Andersen NS**, Pedersen LB, Laurell A, Elonen E, Kolstad A, Boesen AM, Pedersen LM, Lauritzsen GF, Ekanger R, Nilsson-Ehle H, Nordström M, Fredén S, Jerkeman M, Eriksson M, Väärt J, Malmer B, Geisler CH. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. *J Clin Oncol* 2009; **27**: 4365-4370 [PMID: 19652064 DOI: 10.1200/JCO.2008.21.3116]

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MINIREVIEWS

Split liver transplantation: What's unique?

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Abstract

The intraoperative management of split liver transplantation (SLT) has some unique features as compared to routine whole liver transplantations. Only the liver has this special ability to regenerate that confers benefits in survival and quality of life for two instead of one by splitting livers. Primary graft dysfunction may result from small for size syndrome. Graft weight to recipient body weight ratio is significant for both trisegmental and hemiliver grafts. Intraoperative surgical techniques aim to reduce portal hyperperfusion and decrease venous portal pressure. Ischemic preconditioning can be instituted to protect against ischemic reperfusion injury which impacts graft regeneration. Advancement of the technique of SLT is essential as use of split cadaveric grafts expands the donor pool and potentially has an excellent future.

Key words: Graft to recipient body weight ratio; Split liver transplantation; Small for size syndrome; Hemiliver grafts; Portal hyperperfusion

Core tip: The liver has a special ability to regenerate that confers benefits in survival and quality of life for two instead of one by splitting livers. Primary graft dysfunction may result from small for size syndrome. Graft weight to recipient body weight ratio is significant for both trisegmental and hemiliver grafts. Intraoperative surgical techniques aim to reduce portal hyperperfusion and decrease venous portal pressure. Ischemic preconditioning can be instituted to protect against ischemic reperfusion injury which impacts graft regeneration.

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INTRODUCTION

Liver parenchyma is able to regenerate. Additionally, the liver vasculature has lobar and segmental distributions. Thus, the liver is considered to be a double organ and offers benefits in survival and quality of life for two instead of one recipient, by means of dividing or

splitting a graft.

SMALL-FOR-SIZE-SYNDROME

Primary graft dysfunction can result from the use of partial livers despite the absence of other causes such as vascular obstruction or sepsis. This increasingly recognized phenomenon is termed as "small-for-size-syndrome (SFSS)"[1].

The graft exhibits signs of primary graft dysfunction within the first postoperative week. This dysfunction is in absence of other diagnosis such as vascular obstruction, biliary leak, sepsis and immune rejection. Coagulopathy, bilirubinemia and ascitis are typical manifestations of SFSS[2]. SFSS has been studied extensively in both, humans as well as animals.

It has been suggested that portal hyperperfusion of the graft combined with poor venous outflow and reduced arterial flow might cause sinusoidal congestion and endothelial dysfunction, resulting in SFSS. Graft related factors such as graft to recipient body weight ratio < 0.8, impaired venous outflow, steatosis > 30% and prolonged warm/cold ischemia time are positively predictive of SFSS[1].

Another study states that the lower limit of the graft weight to recipient weight ratio can be safely reduced to 0.6% in adult-to-adult living donor liver transplant, if portal pressure control is used[3].

GRAFT ALLOCATION

Though a split liver maybe obtained from a standard criteria donor, splitting it creates two extended criteria grafts, thus increasing the risk of graft failure[4,5]. There are also ethical dilemmas associated with ownership and stewardship of the organs. Is it ethical for a patient to request for an entire organ rather than a split component[6]? There is increased risk of biliary complications with a split liver, and a recipient may wish to thus decline it. Would it be considered coercion if the patient on the top of the waiting were told that if they declined to a splitting of the liver it would be given to the next on the list[6]?

Other considerations include use of the unassigned part of the graft. As per the United Network for Organ Sharing allocation policy, the unassigned part has to be allocated according to the waiting list and cannot be used by the center performing split liver transplantation (SLT). If an incentive is created by allowing the unassigned part of the liver to be retained by the organization, then the number of split livers in the United States will increase[7].

INTRAOPERATIVE FEATURES

The liver can be split *in situ*, on the back table or in the donor hospital before the donor cross-clamp. Notable advantages are a decrease the total ischemia time and increase in the possibility of inter-center sharing. It may take an additional 1-2 h to perform cholangiogram, hilar dissection and parenchymal division. Cholangiogram can be performed to assess surgical splitability[8].

Contrast enhanced computed tomography could be used to perform a virtual resection and volume analysis. Prior to an *in situ* split, one can determine the segmental volume and delineate surgical planes. The anatomy of the hepatic vasculature and biliary structures can be determined. The anticipated graft and remnant liver volumes post resection can be calculated. The severity of portal hypertension can be assessed using a triphasic computer tomographic scan[9]. Liver grafts are then perfused and preserved with Histidine-Tryptophan-Ketoglutarate solution (Custodiol Solution; Essential Pharmaceuticals, Newtown, PA)[8].

Excellent results have been reported with split livers. These are a right tri-segmental graft that includes segments Ⅰ, Ⅳ, Ⅴ, Ⅵ Ⅶ, and Ⅶ; and a left graft consisting of the left lateral lobe including segments Ⅱ and Ⅲ. Pediatric recipients are usually transplanted with the left lateral lobe. The right tri-segmental graft is usually transplanted into an adult recipient[1].

The liver's regeneration capacity is compromised by aging. Therefore acceptable donor age is usually less than 50 years[10]. However, the major challenge in the field of liver transplantation is organ shortage[11-14].

The split liver technique has been further expanded to use two hemiliver grafts: a left lobe and a right lobe, which effectively expands the donor pool. Unfortunately, however, many challenges have surfaced[7,15-17]. Some challenges and unfavorable outcomes have made many transplant centers reluctant to use hemiliver grafts[16,17]. Since the model for end-stage liver disease (MELD) allocation uses the sickest first policy, livers amenable to splitting are most often allocated to patients unsuitable for SLT.

The middle hepatic vein (MHV) is considered "dominant" in drainage of the hemiliver in 27% of cases[18]. A right hepatectomy without the MHV or reconstruction can induce congestion of the paramedian segments Ⅴ and Ⅷ, reducing functional capacity of the graft. When graft survival was analyzed, no significant difference was found with or without harvest of the MHV, as long as a vein interpositional graft was used for anastomosis[19,20]. The MHV primarily drains the right anterior lobe and segment Ⅳ. On the other hand, a meta-analysis discovered that there was better functional recovery of patients who received the right lobes with MHV[21].

It maybe beneficial to maintain a low central venous pressure (CVP) to minimize graft hyperperfusion. Additionally, low CVP decreases backflow bleeding from the hepatic veins and decrease bleeding during parenchymal transection[22]. An analysis stated that patients with a CVP < 5 cm H_2O had a median blood loss of 200 mL, whereas those with CVP > 5 cm H_2O had a median blood loss of 1000 mL[23]. Low CVP facilitates safe dissection of the retro-hepatic vena cava and major hepatic veins and produces decreased postoperative morbidity and reduction of hospital stay[24]. The potential

disadvantages of low CVP anesthesia are chances of perioperative embolism, need for pressor agents and postoperative renal dysfunction.

The partial clamp inserted in the piggyback method allows some venous return, thereby preventing an acute reduction in the preload during inferior vena cava cross clamping. When the patient is unable to tolerate the test cross clamp, it may be prudent to consider venovenous bypass. Presently, in the United States, temporary portocaval shunt is routine practice in 29% of programs, and a low CVP technique is practiced in 54% of centers[25].

The liver weight can be estimated as 2% of donor's body weight, divided into approximate weights of 35% for the left lobe and 65% for the right lobe[8]. It is important to note that since small-for-size grafts require vigorous and immediate hepatocyte proliferation, regeneration is critically required for the success of SLT. In rats, remnant liver of 10% maybe enough. However, in humans, more volume is required for transplantation[26]. Though at three months after partial liver transplantation (50%, 60% size) liver volume slightly exceeds 100% of the standard liver volume in recipients. The graft increase ratio is higher in 50% partial liver transplantation as compared to 30% partial LT[27].

The liver receives approximately 25% of the cardiac output, of which 75% is supplied by the portal vein and the other 25% by the hepatic artery. Hepatic blood flow is reduced by all anesthetic agents and techniques *via* reductions in hepatic blood flow and hepatic oxygen uptake[28].

Intraoperative factors that decrease hepatic blood flow are mechanical ventilation, hypercarbia, positive end expiratory pressure, hypotension, hemorrhage, hypoxemia and surgery. If the decrease in hepatic blood flow is significant, it can result in parenchymal centrilobular necrosis[28]. Etomidate, ketamine and propofol are induction agents. Etomidate decreases hepatic blood flow[29]. Ketamine has little impact on hepatic blood flow. Propofol has a vasodilator effect, ultimately increasing total hepatic blood flow[30,31]. Midazolam has a longer half-life, a reduced clearance, reduced protein binding, a longer duration of action and an enhanced sedative effect. Dexmedetomidine, an alpha-2 adrenergic agonist, with sedative and analgesic properties, is primarily metabolized in the liver[32]. All volatile anesthetics decrease the mean arterial pressure and portal blood flow. Desflurane and sevoflurane have very little or no effect on total hepatic blood flow[33].

The elimination half-life of morphine is prolonged in cirrhosis. The sedative and respiratory depressant effects are exaggerated. Fentanyl has a short duration of action and its elimination is not appreciably altered in patients with cirrhosis[34]. However, unlike fentanyl, plasma clearance and elimination of alfentanil is increased in patients with cirrhosis[35]. Remifentanil is a short acting synthetic opioid that is hydrolyzed by blood and tissue esterases. Its pharmacokinetics is unaltered in patients with severe liver disease[36].

Vecuronium and rocuronium are steroidal muscle relaxants that are metabolized by the liver. In cirrhotic patients, they have decreased clearance, prolonged half-lives, and prolonged neuromuscular blockade. In living donor liver transplantation, requirements of vecuronium were least in the neohepatic phase[37]. Sugammadex can reverse rocuronium rapidly[38]. Cisatracurium undergoes ester hydrolysis and cisatracurium infusions during liver transplantation require increased dosages and result in prolonged recovery[39].

Ischemic preconditioning protects against ischemic reperfusion injury (IRI) in liver transplantation. Lower aspartate aminotransferase levels and significant reduction of moderate-severe hepatocyte swelling is seen[40]. In rat liver, morphine preconditioning protects against IRI. This involves opioid receptors, phosphatidylinositol-3-kinase, and Akt[41]. IP protected against hepatic IRI under isoflurane anesthesia in rats. The mechanism of protection appeared to involve upregulation of Bcl-2 expression resulting in inhibited apoptosis[42]. Human studies have revealed that patients preconditioned with sevoflurane experienced a reduction in peak transaminase levels, an improvement in clinical outcomes, and enhanced benefit in those with steatotic livers. Inducible nitric oxide synthase mRNA was significantly increased in the preconditioned group suggesting a role for nitric oxide[43].

Unfortunately, ischemic preconditioning significantly enhances the extent of split liver graft injury and hinders hepatic regeneration in SFS liver transplant models[44]. Interestingly, rather than IRI, a shift in regeneration ability is more likely to cause liver graft dysfunction and failure following small-for-size transplantation.

Portal hyperperfusion has been cited as one of the causes for SFSS. Thus the most important step is prevention of SSFS through perioperative treatment strategies include reduction of portal blood flow[45]. Lowering the graft perfusion pressure is vital. Hepatic venous congestion due to insufficient vascular orifices or mechanical stenosis and kinking should be prevented[45].

Surgical approaches to prevent SFSS fall into two categories. The first targets portal hyperperfusion by reducing inflow to the graft, including splenic artery modulation and portacaval shunts. The second aims to relieve parenchymal congestion[1]. Adenosine washout maintains the hepatic arterial buffer response (HABR) that maintains constant total blood flow to the liver. Portal blood flow removes adenosine that has a local vasodilator effect on the arterial system[46,47]. However, an exaggerated HABR may contribute to ischemic injury in states of portal hyperfusion, as seen in small for size grafts[48,49]. Prophylactic splenic artery modulation[50,51] produced a significant reduction in portal flow causing a significant reduction in incidence of SFSS.

SFSS grafts are also at least partly associated with persistent elevation of portal venous pressure[52]. Vasopressin infusions have been used in certain insti-

tutions to decrease portal pressures and flow prior to the anhepatic phase[53].

CONCLUSION

The following factors such as changes in recipient and donor selection and matching, changes in allocation and logistics, and improved technical proficiency have influenced outcomes. The risk of graft failure is now similar between split and whole-liver recipients[54].

There are several challenges, and routine application of the hemiliver technique is still controversial, but can achieve excellent outcomes under the model for end-stage liver disease allocation[8]. The 5-year graft survival for hemilivers is comparable to whole livers[8]. Split liver transplantation, which is based on this unique ability of the liver to regenerate, is an excellent idea to increase the donor grafts. Through the expansion of split-liver transplantation, the transplant community might be able to both increase the organ pool and bridge the liver demand-supply gap.

REFERENCES

1 **Gonzalez HD**, Liu ZW, Cashman S, Fusai GK. Small for size syndrome following living donor and split liver transplantation. *World J Gastrointest Surg* 2010; **2**: 389-394 [PMID: 21206720 DOI: 10.4240/wjgs.v2.i12.389]

2 **Dahm F**, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605-2610 [PMID: 16212618 DOI: 10.1111/j.1600-6143.2005.01081.x]

3 **Kaido T**, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, Yagi S, Uemoto S. Lower limit of the graft-to-recipient weight ratio can be safely reduced to 0.6% in adult-to-adult living donor liver transplantation in combination with portal pressure control. *Transplant Proc* 2011; **43**: 2391-2393 [PMID: 21839274 DOI: 10.1016/j.transproceed.2011.05.037]

4 **Feng S**, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, Greenstein SM, Merion RM. Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant* 2006; **6**: 783-790 [PMID: 16539636 DOI: 10.1111/j.1600-6143.2006.01242.x]

5 **Burroughs AK**, Sabin CA, Rolles K, Delvart V, Karam V, Buckels J, O'Grady JG, Castaing D, Klempnauer J, Jamieson N, Neuhaus P, Lerut J, de Ville de Goyet J, Pollard S, Salizzoni M, Rogiers X, Muhlbacher F, Garcia Valdecasas JC, Broelsch C, Jaeck D, Berenguer J, Gonzalez EM, Adam R. 3-month and 12-month mortality after first liver transplant in adults in Europe: predictive models for outcome. *Lancet* 2006; **367**: 225-232 [PMID: 16427491]

6 Split vs whole liver transplantation. OPTN/UNOS Ethics Committee White Paper. Available from: URL: http: //optn.transplant.hrsa.gov/resources/bioethics.asp?index=8

7 **Emre S**, Umman V. Split liver transplantation: an overview. *Transplant Proc* 2011; **43**: 884-887 [PMID: 21486620 DOI: 10.1016/j.transproceed.2011.02.036]

8 **Hashimoto K**, Quintini C, Aucejo FN, Fujiki M, Diago T, Watson MJ, Kelly DM, Winans CG, Eghtesad B, Fung JJ, Miller CM. Split liver transplantation using Hemiliver graft in the MELD era: a single center experience in the United States. *Am J Transplant* 2014; **14**: 2072-2080 [PMID: 25040819 DOI: 10.1111/ajt.12791]

9 **Aucejo FN**, Hashimoto K, Quintini C, Kelly D, Vogt D, Winans C, Eghtesad B, Baker M, Fung J, Miller C. Triple-phase computed tomography and intraoperative flow measurements improve the management of portosystemic shunts during liver transplantation. *Liver Transpl* 2008; **14**: 96-99 [PMID: 18161777]

10 **Schlitt HJ**. Which liver is splitable? In: Rogiers X, Bismuth H, Busuttil RW, DC, Broering D. Azoulay: Split-liver transplantation-theoretical and practical aspects. Darmstadt, Germany: Steinkopff Verlag, 2002: 63

11 **Broering DC**, Topp S, Schaefer U, Fischer L, Gundlach M, Sterneck M, Schoder V, Pothmann W, Rogiers X. Split liver transplantation and risk to the adult recipient: analysis using matched pairs. *J Am Coll Surg* 2002; **195**: 648-657 [PMID: 12437252 DOI: 10.1016/S1072-7515(02)01339-X]

12 **Yersiz H**, Renz JF, Farmer DG, Hisatake GM, McDiarmid SV, Busuttil RW. One hundred in situ split-liver transplantations: a single-center experience. *Ann Surg* 2003; **238**: 496-505; discussion 506-507 [PMID: 14530721 DOI: 10.1097/01.sla.0000089852.29654.72]

13 **Merion RM**, Rush SH, Dykstra DM, Goodrich N, Freeman RB, Wolfe RA. Predicted lifetimes for adult and pediatric split liver versus adult whole liver transplant recipients. *Am J Transplant* 2004; **4**: 1792-1797 [PMID: 15476478 DOI: 10.1111/j.1600-6143.2004.00594.x]

14 **Wilms C**, Walter J, Kaptein M, Mueller L, Lenk C, Sterneck M, Hillert C, Fischer L, Rogiers X, Broering DC. Long-term outcome of split liver transplantation using right extended grafts in adulthood: A matched pair analysis. *Ann Surg* 2006; **244**: 865-872; discussion 872-873 [PMID: 17122611 DOI: 10.1097/01.sla.0000247254.76747.f3]

15 **Ferla F**, Lauterio A, Di Sandro S, Mangoni I, Poli C, Concone G, Cusumano C, Giacomoni A, Andorno E, De Carlis Luciano L. Split-liver full-left full-right: proposal for an operative protocol. *Transplant Proc* 2014; **46**: 2279-2282 [PMID: 25242768 DOI: 10.1016/j.transproceed.2014.07.066]

16 **Giacomoni A**, Lauterio A, Donadon M, De Gasperi A, Belli L, Slim A, Dorobantu B, Mangoni I, De Carlis L. Should we still offer split-liver transplantation for two adult recipients? A retrospective study of our experience. *Liver Transpl* 2008; **14**: 999-1006 [PMID: 18581461 DOI: 10.1002/lt.21466]

17 **Zambelli M**, Andorno E, De Carlis L, Rossi G, Cillo U, De Feo T, Carobbio A, Giacomoni A, Bottino G, Colledan M. Full-right-full-left split liver transplantation: the retrospective analysis of an early multicenter experience including graft sharing. *Am J Transplant* 2012; **12**: 2198-2210 [PMID: 22578214 DOI: 10.1111/j.1600-6143.2012.04071.x]

18 **Radtke A**, Sotiropoulos GC, Sgourakis G, Molmenti EP, Schroeder T, Saner FH, Beckebaum S, Broelsch CE, Broering DC, Malago M. Hepatic venous drainage: how much can we learn from imaging studies? Anatomic-functional classification derived from three-dimensional computed tomography reconstructions. *Transplantation* 2010; **89**: 1518-1525 [PMID: 20410853 DOI: 10.1097/TP.0b013e3181dd6bac]

19 **Kasahara M**, Takada Y, Fujimoto Y, Ogura Y, Ogawa K, Uryuhara K, Yonekawa Y, Ueda M, Egawa H, Tanaka K. Impact of right lobe with middle hepatic vein graft in living-donor liver transplantation. *Am J Transplant* 2005; **5**: 1339-1346 [PMID: 15888039 DOI: 10.1111/j.1600-6143.2005.00817.x]

20 **Adham M**, Dumortier J, Abdelaal A, Sagnard P, Boucaud C, Boillot O. Does middle hepatic vein omission in a right split graft affect the outcome of liver transplantation? A comparative study of right split livers with and without the middle hepatic vein. *Liver Transpl* 2007; **13**: 829-837 [PMID: 17539013 DOI: 10.1002/lt.21133]

21 **Zhang S**, Dong Z, Zhang M, Xia Q, Liu D, Zhang JJ. Right lobe living-donor liver transplantation with or without middle hepatic vein: a meta-analysis. *Transplant Proc* 2011; **43**: 3773-3779 [PMID: 22172845 DOI: 10.1016/j.transproceed.2011.08.100]

22 **Bismuth H**, Castaing D, Garden OJ. Major hepatic resection under total vascular exclusion. *Ann Surg* 1989; **210**: 13-19 [PMID: 2742411 DOI: 10.1097/00000658-198907000-00002]

23 **Jones RM**, Moulton CE, Hardy KJ. Central venous pressure and its effect on blood loss during liver resection. *Br J Surg* 1998; **85**: 1058-1060 [PMID: 9717995 DOI: 10.1046/j.1365-2168.1998.00795.x]

24 **Chen H**, Merchant NB, Didolkar MS. Hepatic resection using intermittent vascular inflow occlusion and low central venous pressure anesthesia improves morbidity and mortality. *J Gastrointest Surg* 2000; **4**: 162-167 [PMID: 10675239 DOI: 10.1016/S1091-255X(00)80052-9]

25 **Schumann R**, Mandell MS, Mercaldo N, Michaels D, Robertson A, Banerjee A, Pai R, Klinck J, Pandharipande P, Walia A. Anesthesia for liver transplantation in United States academic centers: intraoperative practice. *J Clin Anesth* 2013; **25**: 542-550 [PMID: 23994704 DOI: 10.1016/j.jclinane.2013.04.017]

26 **Kiuchi T**, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, Egawa H, Fujita S, Hayashi M, Tanaka K. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation* 1999; **67**: 321-327 [PMID: 10075602]

27 **Fu WY**, Yan JQ, Shi MM, Ma D, Peng CH, Li HW. Suppression of liver regeneration affects hepatic graft survival in small-for-size liver transplantation in rats. *Hepatol Res* 2013; **43**: 300-310 [PMID: 22882432 DOI: 10.1111/j.1872-034X.2012.01071.x]

28 **Dalal A**, Lang JD. Anesthetic considerations for patients with liver disease, hepatic surgery. Abdeldayem H, editor. Available from: URL: http: //www.intechopen.com/books/hepatic-surgery/anesthetic-considerations-for-patients-with-liver-disease

29 **van den Heuvel I**, Wurmb TE, Böttiger BW, Bernhard M. Pros and cons of etomidate--more discussion than evidence? *Curr Opin Anaesthesiol* 2013; **26**: 404-408 [PMID: 23743556 DOI: 10.1097/ACO.0b013e328362a84c]

30 **Meierhenrich R**, Gauss A, Mühling B, Bracht H, Radermacher P, Georgieff M, Wagner F. The effect of propofol and desflurane anaesthesia on human hepatic blood flow: a pilot study. *Anaesthesia* 2010; **65**: 1085-1093 [PMID: 20860555 DOI: 10.1111/j.1365-2044.2010.06504.x]

31 **Suh SJ**, Yim HJ, Yoon EL, Lee BJ, Hyun JJ, Jung SW, Koo JS, Kim JH, Kim KJ, Choung RS, Seo YS, Yeon JE, Um SH, Byun KS, Lee SW, Choi JH, Ryu HS. Is propofol safe when administered to cirrhotic patients during sedative endoscopy? *Korean J Intern Med* 2014; **29**: 57-65 [PMID: 24574834 DOI: 10.3904/kjim.2014.29.1.57]

32 **Wang ZX**, Huang CY, Hua YP, Huang WQ, Deng LH, Liu KX. Dexmedetomidine reduces intestinal and hepatic injury after hepatectomy with inflow occlusion under general anaesthesia: a randomized controlled trial. *Br J Anaesth* 2014; **112**: 1055-1064 [PMID: 24771805 DOI: 10.1093/bja/aeu132]

33 **Kang JG**, Ko JS, Kim GS, Gwak MS, Kim YR, Lee SK. The relationship between inhalational anesthetic requirements and the severity of liver disease in liver transplant recipients according to three phases of liver transplantation. *Transplant Proc* 2010; **42**: 854-857 [PMID: 20430189 DOI: 10.1016/j.transproceed.2010.02.057]

34 **Bosilkovska M**, Walder B, Besson M, Daali Y, Desmeules J. Analgesics in patients with hepatic impairment: pharmacology and clinical implications. *Drugs* 2012; **72**: 1645-1669 [PMID: 22867045 DOI: 10.2165/11635500-000000000-00000]

35 **Höhne C**, Donaubauer B, Kaisers U. [Opioids during anesthesia in liver and renal failure]. *Anaesthesist* 2004; **53**: 291-303 [PMID: 15074320]

36 **Zhang LP**, Yang L, Bi SS, Lu W, Zhang XH, Zhai SD, Duan LP. Population pharmacokinetics of remifentanil in patients undergoing orthotopic liver transplantation. *Chin Med J* (Engl) 2009; **122**: 1032-1038 [PMID: 19493437]

37 **Kim WH**, Joo HS, Ko JS, Gwak MS, Lee SK, Kim GS. Vecuronium requirements according to the operative phase during living donor liver transplantation under desflurane anesthesia. *Transplant Proc* 2013; **45**: 1920-1923 [PMID: 23769073 DOI: 10.1016/j.transproceed.2012.10.064]

38 **Fujita A**, Ishibe N, Yoshihara T, Ohashi J, Makino H, Ikeda M, Setoguchi H. Rapid reversal of neuromuscular blockade by sugammadex after continuous infusion of rocuronium in patients with liver dysfunction undergoing hepatic surgery. *Acta Anaesthesiol Taiwan* 2014; **52**: 54-58 [PMID: 25016508 DOI: 10.1016/j.aat.2014.04.007]

39 **Cammu G**, Bossuyt G, De Baerdemaeker L, Den Blauwen N, Struys M, Mortier E. Dose requirements and recovery profile of an infusion of cisatracurium during liver transplantation. *J Clin Anesth* 2002; **14**: 135-139 [PMID: 11943528 DOI: 10.1016/S0952-8180(01)00370-1]

40 **Franchello A**, Gilbo N, David E, Ricchiuti A, Romagnoli R, Cerutti E, Salizzoni M. Ischemic preconditioning (IP) of the liver as a safe and protective technique against ischemia/reperfusion injury (IRI). *Am J Transplant* 2009; **9**: 1629-1639 [PMID: 19519822 DOI: 10.1111/j.1600-6143.2009.02680.x]

41 **Wang Y**, Wong GT, Man K, Irwin MG. Pretreatment with intrathecal or intravenous morphine attenuates hepatic ischaemia-reperfusion injury in normal and cirrhotic rat liver. *Br J Anaesth* 2012; **109**: 529-539 [PMID: 22745352 DOI: 10.1093/bja/aes209]

42 **Ko JS**, Gwak MS, Kim GS, Shin YH, Ryu S, Kim JS, Kim SJ, Kim ST. The protective effect of ischemic preconditioning against hepatic ischemic-reperfusion injury under isoflurane anesthesia in rats. *Transplant Proc* 2013; **45**: 1704-1707 [PMID: 23769028 DOI: 10.1016/j.transproceed.2012.08.026]

43 **Beck-Schimmer B**, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhan M, Jochum W, Spahn DR, Graf R, Clavien PA. A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. *Ann Surg* 2008; **248**: 909-918 [PMID: 19092335 DOI: 10.1097/SLA.0b013e31818f3dda]

44 **Yao A**, Li X, Pu L, Zhong J, Liu X, Yu Y, Zhang F, Kong L, Sun B, Wang X. Impaired hepatic regeneration by ischemic preconditioning in a rat model of small-for-size liver transplantation. *Transpl Immunol* 2007; **18**: 37-43 [PMID: 17584601]

45 **Umeda Y,** Yagi T, Sadamori H, Fujiwara T. Small-for-size syndromeafter living donor liver transplantation. In: Liver transplantation-technical issues and complications. Abdeldayem H, editor. Available from: URL: http: //www.intechopen.com/books/liver-transplantation-technical-issues-andcomplications/small-for-size-syndrome-after-living-donor-liver-transplantation

46 **Lautt WW**. Regulatory processes interacting to maintain hepatic blood flow constancy: Vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. *Hepatol Res* 2007; **37**: 891-903 [PMID: 17854463 DOI: 10.1111/j.1872-034X.2007.00148.x]

47 **Lautt WW**, Legare DJ, Ezzat WR. Quantitation of the hepatic arterial buffer response to graded changes in portal blood flow. *Gastroenterology* 1990; **98**: 1024-1028 [PMID: 2311859 DOI: 10.1016/0016-5085(90)90029-Z]

48 **Demetris AJ**, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, Tan HP, Shaw-Stiffel T, Boig L, Novelli P, Planinsic R, Fung JJ, Marcos A. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* 2006; **30**: 986-993 [PMID: 16861970 DOI: 10.1097/00000478-200608000-00009]

49 **Smyrniotis V**, Kostopanagiotou G, Kondi A, Gamaletsos E, Theodoraki K, Kehagias D, Mystakidou K, Contis J. Hemodynamic interaction between portal vein and hepatic artery flow in small-for-size split liver transplantation. *Transpl Int* 2002; **15**: 355-360 [PMID: 12122512 DOI: 10.1111/j.1432-2277.2002.tb00178.x]

50 **Troisi R**, Cammu G, Militerno G, De Baerdemaeker L, Decruyenaere J, Hoste E, Smeets P, Colle I, Van Vlierberghe H, Petrovic M, Voet D, Mortier E, Hesse UJ, de Hemptinne B. Modulation of portal graft inflow: a necessity in adult living-donor liver transplantation? *Ann Surg* 2003; **237**: 429-436 [PMID: 12616129 DOI: 10.1097/01.SLA.0000055277.78876.B7]

51 **Umeda Y**, Yagi T, Sadamori H, Matsukawa H, Matsuda H, Shinoura S, Mizuno K, Yoshida R, Iwamoto T, Satoh D, Tanaka N. Effects of prophylactic splenic artery modulation on portal overperfusion and liver regeneration in small-for-size graft. *Transplantation* 2008; **86**: 673-680 [PMID: 18791439 DOI: 10.1097/TP.0b013e318181e02d]

52 **Kiuchi T**, Tanaka K, Ito T, Oike F, Ogura Y, Fujimoto Y, Ogawa K. Small-for-size graft in living donor liver transplantation: how far should we go? *Liver Transpl* 2003; **9**: S29-S35 [PMID: 12942476 DOI: 10.1053/jlts.2003.50198]

53 **Wagener G**, Gubitosa G, Renz J, Kinkhabwala M, Brentjens T, Guarrera JV, Emond J, Lee HT, Landry D. Vasopressin decreases portal vein pressure and flow in the native liver during liver transplantation. *Liver Transpl* 2008; **14**: 1664-1670 [PMID: 18975276 DOI: 10.1002/lt.21602]

54 **Cauley RP**, Vakili K, Fullington N, Potanos K, Graham DA, Finkelstein JA, Kim HB. Deceased-donor split-liver transplantation in adult recipients: is the learning curve over? *J Am Coll Surg* 2013; **217**: 672-684.e1 [PMID: 23978530 DOI: 10.1016/j.jamcollsurg.2013.06.005]

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Obesity and liver transplantation

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Abstract

The percentage of overweight and obese patients (OPs) waiting for a liver transplant continues to increase. Despite the significant advances occurred in bariatric medicine, obesity is still considered a relative contraindication to liver transplantation (LT). The main aim of this review is to appraise the literature on the outcomes of OPs undergoing LT, treatments that might reduce their weight before, during or after surgery, and discuss some of the controversies and limitations of the current knowledge with the intent of highlighting areas where future research is needed.

Key words: Liver transplantation; Bariatric surgery; Obesity; End-stage liver disease; Weight-loss; Access to transplantation

Core tip: The prevalence of obesity in the general population has doubled and the number of obese patients (OPs) affected by end-stage liver disease has increased with the same pace. There is conflicting data on the outcomes of OPs undergoing liver transplantation (LT) and the main aim of this review is to appraise the literature on the outcomes of OPs undergoing LT, treatments that might reduce their weight before, during or after surgery, and discuss some of the controversies and limitations of the current knowledge with the intent of highlighting areas where future research is needed.

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INTRODUCTION

The incidence and prevalence of obesity, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have increased worldwide. In 2010, 35.7% of the adults living in the United States were affected by obesity and the estimated prevalence of NAFLD and NASH were 30% and 12% respectively[1,2].

In the last decade, the indication for liver transplantation (LT) for NASH has risen from 1.2% to 9.7%, and is currently the third most common cause of liver failure and might become the leading indication for LT by 2025[3].

Since the percentage of obese patients (OPs) with end-stage-liver-disease (ESLD) continues to rise, familiarity with the evolving field of bariatric medicine is necessary for transplant specialists. The main objectives of this paper is to review the most recent literature on the treatment options, to discuss some of the implications that obesity has for LT recipients, and finally, to explore current controversies and possible directions for future research.

DEFINITION OF OBESITY

Obesity is defined by the World Health Organization[4] as the presence of excessive body fat that poses health risks, and body mass index (BMI) is the most common metric used by normalizing a person's weight to her/his height. Individuals with a BMI equal or greater than 30 kg/m^2 are defined as obese and individuals with a BMI equal or greater than 40 kg/m^2 are categorized as morbidly obese.

NON-SURGICAL THERAPIES IN CIRRHOTIC PATIENTS

Dieting, physical activity, behavioral therapy, and pharmacotherapy are acceptable but poorly effective options for the treatment of obesity. The Food and Drug Administration has approved orlistat, lorcaserin, and phentermine-topiramate for weight loss but not for cirrhotic patients[5]. Orlistat (Xenical®) acts by blocking gastric and pancreatic lipases and inhibits triglycerides absorption. Locaserin HCl (Belviq®) suppresses the appetite and promotes satiety by acting as an agonist for serotonin receptors in the hypothalamus. Finally, phentermine-topiramate (Qsymia®) decreases appetite by a catecholamine effect in the central nervous system[6].

Medically supervised weight-loss (MSWL) has a low success rate[6-9] as patients fail to maintain their desired weight[10]. Additionally, possible interactions between immunosuppressive medications and drugs used to reduce BMI are unknown[11] and further research is needed before weight-loss medications can be recommended either before or after LT.

BARIATRIC SURGERY

In recent years, the introduction of minimally invasive techniques has considerably reduced the perioperative morbidity and mortality of patients undergoing bariatric surgery (BS)[12]. The Metabolic and BS Accreditation and Quality Improvement Program have created national standards for bariatric programs similarly to what UNOS has done for transplant centers[13] with the subsequent fall of perioperative mortality to 1%[14]. Because of its safety and long-term effectiveness, BS has become the most frequent therapy for non-cirrhotic OPs[15].

BS can be categorized into three main classes: restrictive, mostly restrictive and malabsorptive (Figure 1). Although most of the BS have overlapping effects, restrictive surgeries primarily work by reducing the gastric capacity while malabsorptive surgeries prevent absorption of nutrients.

Among all the BS procedures, adjustable gastric banding (AGB) (Figure 1A) is the least invasive and it is purely restrictive. An adjustable band is positioned at the upper portion of the stomach and connected to a subcutaneous port that allows health care providers to inflate (or deflate) the band with the final goal of reducing the gastric capacity and patients' appetite.

Sleeve gastrectomy (SG), is a restrictive procedure that involves the removal of the majority (60%-70%) of the greater curvature of the stomach, leaving only a sleeve of functioning stomach (Figure 1B). This procedure reduces the gastric volume and the level of ghrelin secreted by the stomach with subsequent decrease of patients' sensation of hunger. Roux-en-Y gastric bypass (RYGB), a mostly restrictive procedure creates a small gastric pouch (approximately 5% of the original gastric volume) and re-routes 100-150 cm of proximal intestine (Figure 1C). Duodenal switch (DS), also known as biliopancreatic diversion, combines malabsortive and restrictive effects as a partial gastrectomy and extensive re-routing of the small intestine are performed simultaneously (Figure 1D). The common intestinal channel where food can be absorbed is reduced to only 75-150 cm and is currently performed in selected groups of morbidly OPs accounting for only 1% of all BS performed annually in the United States.

BENEFITS OF BS

Pontiroli *et al*[16] performed a systematic review and meta-analysis of eight trials involving 44022 OPs and found that BS reduced their risk of death due to metabolic syndrome (MS) (OR = 0.55; $P < 0.05$). Similar results were reported by Johnson *et al*[17]. Schauer *et al*[18] analyzed 150 patients randomized to BS *vs* best medical therapy for the treatment of type Ⅱ diabetes (T2DM). At 12-mo, the glycemic control was significantly better in patients who underwent BS. After 3-years, the target HbA1c level was achieved in 5% of the medical group *vs* 38% in patients who underwent RYGB and 24% in the SG group. A systematic review and meta-analysis of 6587 patients[19], found that for every five-point drop in BMI, the risk reductions for T2DM, hypertension, and dyslipidemia were 33%, 27%, and 20%, respectively. Similar results were reported in another systematic review of 22092 patients[20] where BS was associated with improvement or complete resolution of T2DM (86% of patients), dyslipidemia (70%), hypertension (78%), and obstructive sleep

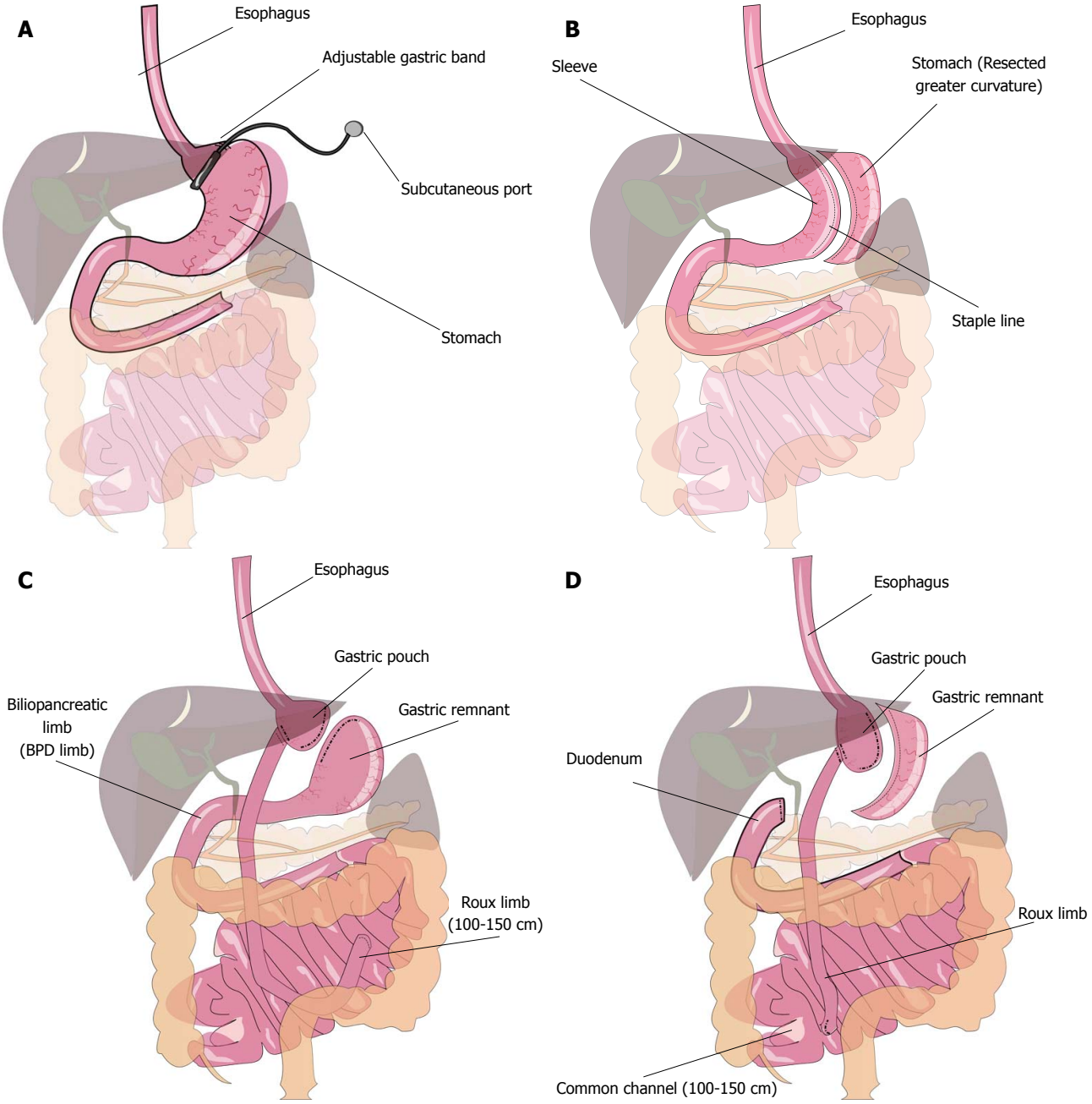

Figure 1 Types of bariatric procedures. A: Adjustable gastric banding; B: Sleeve gastrectomy; C: Roux-en-Y gastric bypass; D: Duodenal switch.

apnea (86%).

OPS WAITING FOR LT: SHOULD THEY UNDERGO BARIATRIC TREATMENT?

Theoretically, OPs with ESLD should benefit from losing weight as it reduces their risk for cardiovascular diseases, T2DM, dyslipidemia, obstructive sleep apnea *etc.* Additionally, OPs on the list for LT might improve their chance of being transplanted as a recent analysis of the United Network for Organ Sharing (UNOS) data[21] has shown that their likelihood of being transplanted was lower in comparison to normal weight individuals. One of the possible explanations is that transplant programs might decline surgery to obese candidates as they are at higher risk for perioperative complications[22] and have lower survival rates in comparison to normal weight patients[3,23]. Although there are some legitimate concerns, declining LT to OPs goes against the principle of fairness, as OPs who undergo LT have a significant survival advantage in comparison to OPs who remain on the waiting list and are not transplanted[24].

OUTCOMES OF OPS UNDERGOING LT

LaMattina *et al*[25] analyzed the perioperative morbidity of 813 LT patients between 1997 and 2008, and found that OPs had prolonged mean operative time (class Ⅰ obesity: 7.7 h, P = 0.009; class Ⅱ obesity: 7.9 h, P = 0.008;

class Ⅲ obesity: 8.2 h, $P = 0.003$ *vs* normal weight: 7.2 h), ICU stay (Class Ⅱ obesity: 4.1 d *vs* 2.6 d; $P = 0.04$), increased need for transfusions (class Ⅰ obesity: 15 units, $P = 0.005$; class Ⅱ obesity: 16 units, $P = 0.005$; class Ⅲ obesity: 15 units, $P = 0.08$ *vs* normal weight: 11 units), higher incidence of infections (HR 7.21, CI: 1.6-32.4, $P = 0.01$), biliary complications requiring intervention (Class Ⅱ obesity: HR 2.04, CI: 1.27-3.3, $P = 0.003$) and, more importantly, decreased patient (Class Ⅱ obesity: HR 1.82, CI: 1.09-3.01, $P = 0.02$) and graft survivals (Class Ⅱ obesity: HR 1.62, CI: 1.02-2.65, $P = 0.04$). In another study of 73538 LT recipients the overall survival was significantly lower in BMI less than 18.5 and higher than 40, compared to a control group[26]. Death in underweight patients was due to hemorrhagic ($P < 0.002$) and cerebrovascular ($P < 0.04$) complications, while infectious complications and cancer were the most common causes of demise in severely obese group ($P = 0.02$)[26]. Nair *et al*[22] analyzed the UNOS database on 18172 LT patients transplanted between 1988 and 1996 and found that primary graft dysfunction, perioperative mortality at 1, 2, and 5-years were significantly higher in the morbidly obese group due to cardiovascular adverse events. Similar outcomes were reported in 1325 obese LT recipients[27] from the United Kingdom where they had increased morbidity due to infectious complications, longer ICU and hospital stay in comparison to normal weight patients.

However, other studies suggested that higher BMI should not be considered an absolute contraindication to LT[24,28]. In 230 LT patients stratified into a lean group (BMI 20-26 kg/m^2) and an obese group (BMI > 38 kg/m^2), no significant differences were found except that at 3-year follow-up, the obese group had a higher risk of developing MS (46% in obese *vs* 21% in lean patients, OR 4.76; CI: 1.66-13.7, $P < 0.001$). Similar results were noted in a retrospective study of 25647 LT waitlist patients. In comparison to being on waitlist, all subgroups of BMI had survival advantage ($P < 0.0001$) with LT. Similar outcomes were noted by Conzen *et al*[23] in a single-center study of 785 patients. Three-year patient and graft survival were similar in all groups of BMI, while 5-year patient (51.3% *vs* 78.8%; $P < 0.01$) and graft (49% *vs* 75.8%; $P < 0.02$) survival were significantly reduced in morbidly obese *vs* non-OPs.

POSSIBLE ADVANTAGES OF BS FOR OPS REQUIRING A LIVER TRANSPLANT

The potential benefits of BS for patients in need of a LT have never been studied by randomized trials. Theoretically, weight-loss interventions would reduce their risk of suboptimal outcomes and may prevent the development of MS and recurrent NASH after LT. On the other hand, perioperative morbidity and mortality risks might be too high to justify any surgery to reduce their BMI.

THE PROS AND CONS OF DIFFERENT BARIATRIC SURGERIES

AGB is a relatively simple procedure that does not require the rerouting of the gastrointestinal tract and maintains the endoluminal access to the biliary system for endoscopic treatment of biliary complications that can occur after LT. AGB has no risks of anastomotic dehiscence and it is reversible (Table 1). The main drawback of AGB is the presence of a foreign body that could become infected and cause long-term complications from slippage, prolapse, port-site infection and erosion into the stomach with potential serious consequences in immunocompromised patients. Other potential issues with AGB are that the band is positioned near the gastroesophageal junction where varices from chronic portal hypertension develop, and the band could prevent access to the supraceliac aorta for arterial reconstructions during LT if necessary.

RYGB and DS are more effective than AGB, but have significantly higher perioperative risks of anastomotic leaks, obstructions, marginal ulcers, malabsorption of immunosuppression medications, loss of endoscopic access to the biliary system and are contraindicated for patients who need a Roux-limb for their biliary reconstruction.

In recent years, SG has been viewed as a good compromise as it has lower perioperative risks in comparison to RYGB or DS[29], maintains direct access to the biliary system, it is unlikely to cause malabsorption of immunosuppression medications[30] and provides a gradual and sustained weight-loss[9,31,32].

TIMING FOR BS

Before transplant

The rationale for performing BS prior to LT would be to optimize patients' medical condition before surgery or to bring patients' BMI within the range considered acceptable by some transplant centers.

However, BS performed before LT might delay transplant surgery due to the time necessary to achieve the desired BMI or to the development of perioperative complications. Another drawback of BS before LT is that recipients undergo two separate operations and two hospitalizations with associated increased financial costs, stress, and pain.

Although no randomized controlled trials have ever been conducted to test whether BS is beneficial for OP requiring LT, case reports and observational studies have described the feasibility of BS either pre-, during or post-LT. Lin *et al*[33] published a retrospective review of all SG performed in liver (20 patients) and kidney transplant candidates (6 patients) between 2006 and 2012. The mean excess weight-loss (EWL) at 1, 3, and 12 mo was 17%, 26%, and 50% respectively without any perioperative death. Six cases (16%) experienced postoperative complications, including superficial wound infections, staple line leak, bleeding requiring

Table 1 Summary of advantages and disadvantages of different categories of bariatric surgeries in the context of liver transplantation

Procedure	Category	Description	(%) Excess weight loss	Pros	Cons
Adjustable gastric banding	Restrictive	Silicone band placed at the upper portion of the stomach	40-50	Minimally invasive, adjustable, reversible, removable, access to biliary tree is maintained	Foreign body placement, relatively longer duration for weight-loss, long-term potential complications of band erosion, pouchitis, pouch enlargement, gastric prolapse, slippage and flipped port, tubing breakage, malfunction of the device, port site infections
Sleeve gastrectomy	Restrictive	Removal of greater part of greater curvature of the stomach	50-60	Maintains gastric function with direct access to biliary tree, has better tolerance of oral/medications intake and absorption	Long staple-line on the stomach with a potential for bleeding and gastroinstestinal leak
Roux-en-Y gastric bypass	Mostly restrictive	Creation of gastric pouch and rerouting of intestine	70	Combined restrictive and malabsorptive procedure, resolution of comorbidities is relatively quicker with higher proportion of weight-loss	Relatively higher significant perioperative complications, intolerance to oral consumption, and absorption of medications, loss of direct access to biliary tree and remnant stomach, can lead to excessive weight-loss, higher likelihood of malnourishment
Duodenal switch	Malabsorptive	Subtotal gastrectomy with a very short common channel	80		

Percentage of excess weight loss = [(preoperative weight - weight at follow-up)/(preoperative weight - ideal body weight)] × 100.

transfusion, transient encephalopathy and renal insufficiency. All these patients became transplantable candidates by meeting institutional BMI requirements at 12 mo and the authors concluded that SG is relatively safe and effective.

Similar conclusions were drawn by Takata *et al*[34] who evaluated the effect of BS in end-stage liver, kidney, and lung disease in 15 OPs who were considered unsuitable for transplantation. Mean EWL at or after 9 mo was 61%, 33%, and 61% respectively. Obesity-associated comorbidities improved in all patients and, except for two individuals (13%) who suffered from perioperative complications, no deaths occurred after surgery. More importantly, 93% of patients became transplant candidates by meeting the institutional requirements on BMI. These authors concluded that laparoscopic RYGB and SG is safe and improves the candidacy for transplantation. With gain in experience in cadaveric LT and BS, feasibility is being evaluated also in living donor LT. Taneja *et al*[35] published a successful outcome of SG in a patient with BMI of 55.6 and NASH undergoing living donor LT.

After transplant

The main rationale for performing BS after LT would be to prevent the recurrence of MS and NASH and improve survival by reducing obesity related comorbidities[36]. In a recent publication, Duchini *et al*[37] described two patients who were successfully treated by RYGB for severe graft dysfunction due to recurrent NASH.

However, BS after LT comes with the risk of dealing with severe adhesions, wound complications and anastomotic or staple lines dehiscences due to the use of steroids and/or m-TOR inhibitors. Despite these potential drawbacks, Lin *et al*[38] published a pilot study on the safety and feasibility of SG in nine obese LT recipients with the intent of improving steroid-induced diabetes, steatohepatitis, and MS. Postoperative complications occurred in three patients (33%) who developed mesh infection in a concurrent ventral hernia repair, bile leak requiring drainage and one patient who underwent reoperation for dysphagia. At 6 mo, 55% EWL was achieved without graft rejection and the authors concluded that SG does not adversely affect LT function. On the other hand, some technical challenges associated with BS after LT were reported by Tichansky *et al*[39] who described major adhesions with complete obliteration of the gastrohepatic space during a successful laparoscopic RYGB after LT for a patient with a BMI of 54 kg/m^2.

During LT

Combining BS and LT could theoretically minimize delays, hospital stay and reduce patients' overall pain as the same incision can be used for both operations. However, one of the biggest trade-offs is that the operation for LT will take longer and that patients might suffer from more severe complications due to the increased complexity of the procedure.

Campsen *et al*[40] performed a successful simultaneous LT and AGB and reported that at 6 mo, patients' BMI went from 42 kg/m^2 to 34 kg/m^2 with 45% EWL and resolution of T2DM, hypertension and osteoarthritis. In 2013, Heimbach *et al*[41] published their experience of BS in OPs (BMI $\geqslant$ 35) undergoing LT. OPs with a BMI $\geqslant$ 35 were divided into two groups. Patients who successfully completed MSWL underwent LT (n = 37) alone. Seven patients who failed MSWL underwent simultaneous LT and SG (n = 7). In patients who underwent LT alone, weight-regain (BMI > 35) was noted in 21 of 34 patients (61%), post-transplant diabetes in 12 patients (35%),

steatosis in 7 (20%), graft losses and deaths in 3 (8%). In the group of patients who underwent simultaneous LT and SG (n = 7), all maintained their weight-loss, one had a gastrointestinal leak from the staple-line (14%) and one had excessive weight-loss. Although the majority of patients who did not undergo BS achieved some weight-loss with a non-surgical approach, most regained weight within a mean follow-up of 33 mo. On the other hand, patients treated with combination of SG and LT achieved effective and sustained weight-loss and fewer metabolic complications over a mean follow-up of 17 mo.

CONCLUSION

The obesity epidemic is having a significant impact on the field of transplantation as two-thirds of the adult population in the United States is overweight. Although OPs undergoing LT might experience short and long term-outcomes inferior to patients with normal BMI, their survival with LT is superior to best supportive care. Therefore, their exclusion from LT would violate the idea of fairness and should be challenged. Since medical therapies are relatively ineffective, BS might play a more distinct role in the future of transplantation but there are no well-designed studies on the role of BS in this population. Currently, only low quality evidence (Level 4 and 3b)[42] has shown that BS can be done either prior, during or after LT. However, the number of publications is small, and except for a few case-series, there are no studies that have systematically compared OPs treated with MSWL *vs* BS *vs* no treatment. Similarly, there is lack of data on the best timing of BS (prior to LT, during or after LT) or which type of BS (AGB *vs* RYGB *vs* SG *vs* DS) should be performed.

In summary, the number of OPs requiring LT is rising. To maximize short and long-term outcomes of OPs undergoing LT, prospective studies should be designed to identify if there are benefits from weight-loss treatments and if so, what interventions should be used and when they should be instituted.

REFERENCES

1 **Phongsamran PV**, Kim JW, Cupo Abbott J, Rosenblatt A. Pharmacotherapy for hepatic encephalopathy. *Drugs* 2010; **70**: 1131-1148 [PMID: 20518580 DOI: 10.2165/10898630-000000000-00000]

2 **Ogden CL**, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011-2012. *NCHS Data Brief* 2013; **(131)**: 1-8 [PMID: 24152742]

3 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

4 **Kopelman PG**. Obesity as a medical problem. *Nature* 2000; **404**: 635-643 [PMID: 10766250]

5 **Thurairajah PH**, Syn WK, Neil DA, Stell D, Haydon G. Orlistat (Xenical)-induced subacute liver failure. *Eur J Gastroenterol Hepatol* 2005; **17**: 1437-1438 [PMID: 16292105 DOI: 10.1097/01.meg.0000187680.53389.88]

6 **Yanovski SZ**, Yanovski JA. Long-term drug treatment for obesity: a systematic and clinical review. *JAMA* 2014; **311**: 74-86 [PMID: 24231879 DOI: 10.1001/jama.2013.281361]

7 **Halperin F**, Ding SA, Simonson DC, Panosian J, Goebel-Fabbri A, Wewalka M, Hamdy O, Abrahamson M, Clancy K, Foster K, Lautz D, Vernon A, Goldfine AB. Roux-en-Y gastric bypass surgery or lifestyle with intensive medical management in patients with type 2 diabetes: feasibility and 1-year results of a randomized clinical trial. *JAMA Surg* 2014; **149**: 716-726 [PMID: 24899464 DOI: 10.1001/jamasurg.2014.514]

8 **Picot J**, Jones J, Colquitt JL, Gospodarevskaya E, Loveman E, Baxter L, Clegg AJ. The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. *Health Technol Assess* 2009; **13**: 1-190, 215-357, iii-iv [PMID: 19726018 DOI: 10.3310/hta13410]

9 **Colquitt JL**, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. *Cochrane Database Syst Rev* 2014; **8**: CD003641 [DOI: 10.1002/14651858.cd003641.pub4]

10 **Colquitt JL**, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. *Cochrane Database Syst Rev* 2014; **8**: CD003641 [PMID: 25105982 DOI: 10.3310/hta15020]

11 **Schnetzler B**, Kondo-Oestreicher M, Vala D, Khatchatourian G, Faidutti B. Orlistat decreases the plasma level of cyclosporine and may be responsible for the development of acute rejection episodes. *Transplantation* 2000; **70**: 1540-1541 [PMID: 11118104 DOI: 10.1097/00007890-200011270-00025]

12 **Nguyen NT**, Nguyen B, Shih A, Smith B, Hohmann S. Use of laparoscopy in general surgical operations at academic centers. *Surg Obes Relat Dis* 2013; **9**: 15-20 [PMID: 22892343 DOI: 10.1016/j.soard.2012.07.002]

13 **Morton J**. The first metabolic and bariatric surgery accreditation and quality improvement program quality initiative: decreasing readmissions through opportunities provided. *Surg Obes Relat Dis* 2014; **10**: 377-378 [PMID: 24951058 DOI: 10.1016/j.soard.2014.02.036]

14 **Athyros VG**, Tziomalos K, Karagiannis A, Mikhailidis DP. Cardiovascular benefits of bariatric surgery in morbidly obese patients. *Obes Rev* 2011; **12**: 515-524 [PMID: 21348922 DOI: 10.1111/j.1467-789X.2010.00831.x]

15 **Buchwald H**, Oien DM. Metabolic/bariatric surgery worldwide 2011. *Obes Surg* 2013; **23**: 427-436 [PMID: 23338049 DOI: 10.1007/s11695-012-0864-0]

16 **Pontiroli AE**, Morabito A. Long-term prevention of mortality in morbid obesity through bariatric surgery. a systematic review and meta-analysis of trials performed with gastric banding and gastric bypass. *Ann Surg* 2011; **253**: 484-487 [PMID: 21245741 DOI: 10.1097/SLA.0b013e31820d98cb]

17 **Johnson RJ**, Johnson BL, Blackhurst DW, Bour ES, Cobb WS, Carbonell AM, Lokey JS, Scott JD. Bariatric surgery is associated with a reduced risk of mortality in morbidly obese patients with a history of major cardiovascular events. *Am Surg* 2012; **78**: 685-692 [PMID: 22643265]

18 **Schauer PR**, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. *N Engl J Med* 2014; **370**: 2002-2013 [PMID: 24679060 DOI: 10.1056/NEJMoa1401329]

19 **Ricci C**, Gaeta M, Rausa E, Macchitella Y, Bonavina L. Early impact of bariatric surgery on type II diabetes, hypertension, and hyperlipidemia: a systematic review, meta-analysis and meta-regression on 6,587 patients. *Obes Surg* 2014; **24**: 522-528 [PMID: 24214202]

20 **Buchwald H**, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, Schoelles K. Bariatric surgery: a systematic review and meta-analysis. *JAMA* 2004; **292**: 1724-1737 [PMID: 15479938 DOI: 10.1001/jama.292.14.1724]

21 **Segev DL**, Thompson RE, Locke JE, Simpkins CE, Thuluvath PJ, Montgomery RA, Maley WR. Prolonged waiting times for liver transplantation in obese patients. *Ann Surg* 2008; **248**: 863-870 [PMID: 18948816 DOI: 10.1097/SLA.0b013e31818a01ef]

22 **Nair S**, Verma S, Thuluvath PJ. Obesity and its effect on survival

in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002; **35**: 105-109 [PMID: 11786965 DOI: 10.1053/jhep.2002.30318]

23 **Conzen KD**, Vachharajani N, Collins KM, Anderson CD, Lin Y, Wellen JR, Shenoy S, Lowell JA, Doyle MB, Chapman WC. Morbid obesity in liver transplant recipients adversely affects longterm graft and patient survival in a single-institution analysis. *HPB* (Oxford) 2015; **17**: 251-257 [PMID: 25322849 DOI: 10.1111/hpb.12340]

24 **Perez-Protto SE**, Quintini C, Reynolds LF, You J, Cywinski JB, Sessler DI, Miller C. Comparable graft and patient survival in lean and obese liver transplant recipients. *Liver Transpl* 2013; **19**: 907-915 [PMID: 23744721 DOI: 10.1002/lt.23680]

25 **LaMattina JC**, Foley DP, Fernandez LA, Pirsch JD, Musat AI, D'Alessandro AM, Mezrich JD. Complications associated with liver transplantation in the obese recipient. *Clin Transplant* 2012; **26**: 910-918 [PMID: 22694047 DOI: 10.1111/j.1399-0012.2012.01669.x]

26 **Dick AA**, Spitzer AL, Seifert CF, Deckert A, Carithers RL, Reyes JD, Perkins JD. Liver transplantation at the extremes of the body mass index. *Liver Transpl* 2009; **15**: 968-977 [PMID: 19642131 DOI: 10.1002/lt.21785]

27 **Hakeem AR**, Cockbain AJ, Raza SS, Pollard SG, Toogood GJ, Attia MA, Ahmad N, Hidalgo EL, Prasad KR, Menon KV. Increased morbidity in overweight and obese liver transplant recipients: a single-center experience of 1325 patients from the United Kingdom. *Liver Transpl* 2013; **19**: 551-562 [PMID: 23408499 DOI: 10.1002/lt.23618]

28 **Pelletier SJ**, Maraschio MA, Schaubel DE, Dykstra DM, Punch JD, Wolfe RA, Port FK, Merion RM. Survival benefit of kidney and liver transplantation for obese patients on the waiting list. *Clin Transpl* 2003: 77-88 [PMID: 15387099]

29 **Leyba JL**, Llopis SN, Aulestia SN. Laparoscopic Roux-en-Y gastric bypass versus laparoscopic sleeve gastrectomy for the treatment of morbid obesity. a prospective study with 5 years of follow-up. *Obes Surg* 2014; **24**: 2094-2098 [PMID: 25012769 DOI: 10.1007/s11695-014-1365-0]

30 **Gumbs AA**, Gagner M, Dakin G, Pomp A. Sleeve gastrectomy for morbid obesity. *Obes Surg* 2007; **17**: 962-969 [PMID: 17894158 DOI: 10.1007/s11695-007-9151-x]

31 **Zhang Y**, Wang J, Sun X, Cao Z, Xu X, Liu D, Xin X, Qin M. Laparoscopic sleeve gastrectomy versus laparoscopic Roux-en-Y gastric bypass for morbid obesity and related comorbidities: a meta-analysis of 21 studies. *Obes Surg* 2015; **25**: 19-26 [PMID: 25092167 DOI: 10.1007/s11695-014-1303-1]

32 **Catheline JM**, Fysekidis M, Bachner I, Bihan H, Kassem A, Dbouk R, Bdeoui N, Boschetto A, Cohen R. Five-year results of sleeve gastrectomy. *J Visc Surg* 2013; **150**: 307-312 [PMID: 24060743 DOI: 10.1016/j.jviscsurg.2013.08.008]

33 **Lin MY**, Tavakol MM, Sarin A, Amirkiai SM, Rogers SJ, Carter JT, Posselt AM. Laparoscopic sleeve gastrectomy is safe and efficacious for pretransplant candidates. *Surg Obes Relat Dis* 2013; **9**: 653-658 [PMID: 23701857 DOI: 10.1016/j.soard.2013.02.013]

34 **Takata MC**, Campos GM, Ciovica R, Rabl C, Rogers SJ, Cello JP, Ascher NL, Posselt AM. Laparoscopic bariatric surgery improves candidacy in morbidly obese patients awaiting transplantation. *Surg Obes Relat Dis* 2008; **4**: 159-164; discussion 164-165 [PMID: 18294923 DOI: 10.1016/j.soard.2007.12.009]

35 **Taneja S**, Gupta S, Wadhawan M, Goyal N. Single-lobe living donor liver transplant in a morbidly obese cirrhotic patient preceded by laparoscopic sleeve gastrectomy. *Case Rep Transplant* 2013; **2013**: 279651 [PMID: 24386588 DOI: 10.1155/2013/279651]

36 **Al-Nowaylati AR**, Al-Haddad BJ, Dorman RB, Alsaied OA, Lake JR, Chinnakotla S, Slusarek BM, Sampson BK, Ikramuddin S, Buchwald H, Leslie DB. Gastric bypass after liver transplantation. *Liver Transpl* 2013; **19**: 1324-1329 [PMID: 24039124 DOI: 10.1002/lt.23734]

37 **Duchini A**, Brunson ME. Roux-en-Y gastric bypass for recurrent nonalcoholic steatohepatitis in liver transplant recipients with morbid obesity. *Transplantation* 2001; **72**: 156-159 [PMID: 11468551 DOI: 10.1097/00007890-200107150-00029]

38 **Lin MY**, Tavakol MM, Sarin A, Amirkiai SM, Rogers SJ, Carter JT, Posselt AM. Safety and feasibility of sleeve gastrectomy in morbidly obese patients following liver transplantation. *Surg Endosc* 2013; **27**: 81-85 [PMID: 22752278 DOI: 10.1007/s00464-012-2410-5]

39 **Tichansky DS**, Madan AK. Laparoscopic Roux-en-Y gastric bypass is safe and feasible after orthotopic liver transplantation. *Obes Surg* 2013; **15**: 1481-1486 [PMID: 16354531 DOI: 10.1381/096089205774859164]

40 **Campsen J**, Zimmerman M, Shoen J, Wachs M, Bak T, Mandell MS, Kam I. Adjustable gastric banding in a morbidly obese patient during liver transplantation. *Obes Surg* 2008; **18**: 1625-1627 [PMID: 18704606 DOI: 10.1007/s11695-008-9633-5]

41 **Heimbach JK**, Watt KD, Poterucha JJ, Ziller NF, Cecco SD, Charlton MR, Hay JE, Wiesner RH, Sanchez W, Rosen CB, Swain JM. Combined liver transplantation and gastric sleeve resection for patients with medically complicated obesity and end-stage liver disease. *Am J Transplant* 2013; **13**: 363-368 [PMID: 23137119 DOI: 10.1111/j.1600-6143.2012.04318.x]

42 **Sackett DL**. Evidence-based medicine. *Spine* (Phila Pa 1976) 1998; **23**: 1085-1086 [PMID: 9615357 DOI: 10.1097/00007632-199805150-00001]

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ORIGINAL ARTICLE

Retrospective Study

Role of steroid maintenance in sensitized kidney transplant recipients

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Abstract

AIM: To evaluate whether there is a threshold sensitization level beyond which benefits of chronic steroid maintenance (CSM) emerge.

METHODS: Using Organ Procurement and Transplant Network/United Network of Organ Sharing database, we compared the adjusted graft and patient survivals for CSM *vs* early steroid withdrawal (ESW) among patients who underwent deceased-donor kidney (DDK) transplantation from 2000 to 2008 who were stratified by peak-panel reactive antibody (peak-PRA) titers (0%-30%, 31%-60% and > 60%). All patients received perioperative induction therapy and maintenance immunosuppression based on calcineurin inhibitor (CNI) and mycophenolate mofetil (MMF).

RESULTS: The study included 42851 patients. In the 0%-30% peak-PRA class, adjusted over-all graft-failure (HR 1.11, 95%CI: 1.03-1.20, P = 0.009) and patient-death (HR 1.29, 95%CI: 1.16-1.43, P < 0.001) risks were higher and death-censored graft-failure risk (HR 1.06, 95%CI: 0.98-1.14, P = 0.16) similar for CSM (n = 25218) *vs* ESW (n = 7399). Over-all (HR 1.04, 95%CI: 0.85-1.28, P = 0.70) and death-censored (HR 0.97, 95%CI: 0.78-1.21, P = 0.81) graft-failure risks were similar and patient-death risk (HR 1.39, 95%CI: 1.03-1.87, P = 0.03) higher for CSM (n = 3495) *vs* ESW (n = 850) groups for 31%-60% peak-PRA class. In the > 60% peak-PRA class, adjusted overall graft-failure (HR 0.90, 95%CI: 0.76-1.08, P = 0.25) and patient-death (HR 0.92, 95%CI: 0.71-1.17, P = 0.47) risks were similar and death-censored graft-failure risk lower (HR 0.84, 95%CI: 0.71-0.99, P = 0.04) for CSM (n = 4966)

vs ESW (*n* = 923).

CONCLUSION: In DDK transplant recipients who underwent perioperative induction and CNI/MMF maintenance, CSM appears to be associated with increased risk for death with functioning graft in minimally-sensitized patients and improved death-censored graft survival in highly-sensitized patients.

Key words: Sensitization; Kidney transplantation; Graft survival; Steroid withdrawal; Older kidney transplant recipients

Core tip: This study critically evaluated the role of steroid maintenance in kidney transplant recipients (KTR) based on the level of sensitization by utilizing the Organ Procurement and Transplant Network/United Network of Organ Sharing database. In the multivariate model, we found an association between increased risk for death with functioning graft and steroid maintenance in KTRs who had peak-panel reactive antibody < 30% and received perioperative induction therapy followed by calcineurin inhibitor/mycophenolate mofetil maintenance. On the other hand, steroid maintenance was associated with improved death-censored graft survival without adversely impacting patient survival in KTRs with a peak PRA > 60%. No benefits of steroid maintenance were observed in older KTRs regardless of level of sensitization. These finding have clinical relevance and should be further evaluated in randomized clinical trials.

Sureshkumar KK, Marcus RJ, Chopra B. Role of steroid maintenance in sensitized kidney transplant recipients. *World J Transplant* 2015; 5(3): 102-109 Available from: URL: http://www.wjgnet.com/2220-3230/full/v5/i3/102.htm DOI: http://dx.doi.org/10.5500/wjt.v5.i3.102

INTRODUCTION

Historically, corticosteroid has enjoyed a pivotal role in maintenance immunosuppression in kidney transplant recipients (KTRs). Chronic steroid therapy can worsen hypertension and dyslipidemia, as well as contribute to the development of new onset diabetes mellitus, all risk factors for cardiovascular disease. Steroid therapy makes patients prone to infections and accelerated bone loss. Routine use of induction therapy along with the availability of more potent immunosuppressive agents such as tacrolimus and mycophenolate mofetil (MMF) has enabled transplant professionals to utilize early steroid withdrawal (ESW) in KTRs. The concern with ESW includes increased risk of acute rejection which might adversely impact graft outcomes. Current data suggest that corticosteroids could be discontinued safely during the first week after transplantation in patients who are at low immunological risk and receive induction therapy[1]. Studies of ESW have shown outcomes comparable to steroid maintenance regimens[2-11]. A recent registry analysis showed that the percentage of KTRs discharged from the initial transplant admission on a steroid-free maintenance immunosuppression increased from 3.7% in the year 2000 to 32.5% as of 2006[12].

Patients who develop anti-human leukocyte antigen (anti-HLA) antibodies due to factors such as prior pregnancy, blood transfusion or previous transplant rejection are generally considered immunologically high risk and many transplant centers keep these sensitized patients on a steroid maintenance immunosuppressive protocol in the hopes of reducing the risk for acute rejection. It is not clear whether there is a threshold level of sensitization at which the beneficial effects of steroid maintenance begin to emerge in such patients. We aimed to compare the outcomes for steroid *vs* no steroid addition to a calcineurine inhibitor (CNI)/MMF based regimen in patients who underwent deceased donor kidney (DDK) transplantation after receiving perioperativevinduction therapy and stratified by the level of peak panel reactive antibody (peak-PRA) titer.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board and was performed in accordance with the ethical standards laid down by the Declaration of Helsinki as well as Declaration of Istanbul. Using Organ Procurement and Transplant Network (OPTN)/United Network of Organ Sharing database, we identified patients ⩾ 18 years who underwent a DDK transplantation between January 1, 2000 and December 31, 2008 after receiving antibody induction therapy with rabbit- antithymocyte globulin (r-ATG), alemtuzumab or an interleukine-2 receptor blocker agent (IL-2R, basiliximab or daclizumab) and discharged on a CNI/MMF based maintenance immunosuppression regimen with or without steroids. Prednisone is generally the steroid used for maintenance therapy. Patients were divided into three groups based on the reported peak-PRA: 0%-30%, 31%-60% and > 60%. Under each peak-PRA category, patients were further divided into two groups: Those who underwent ESW before the hospital discharge (ESW group) and those who were discharged on steroid maintenance. The latter group was designated as chronic steroid maintenance (CSM) group. This was an intention-to-treat analysis using the maintenance immunosuppression regimen at the time of discharge from the initial transplant hospitalization as the basis for defining the groups. Changes in maintenance immunosuppression that occurred after initial discharge were not used to classify study subjects. We did not include patients who received live donor kidneys, multi-organ transplants, no induction, more than one induction, induction therapy with a different agent or maintenance other than CNI/MMF based regimen in the analysis.

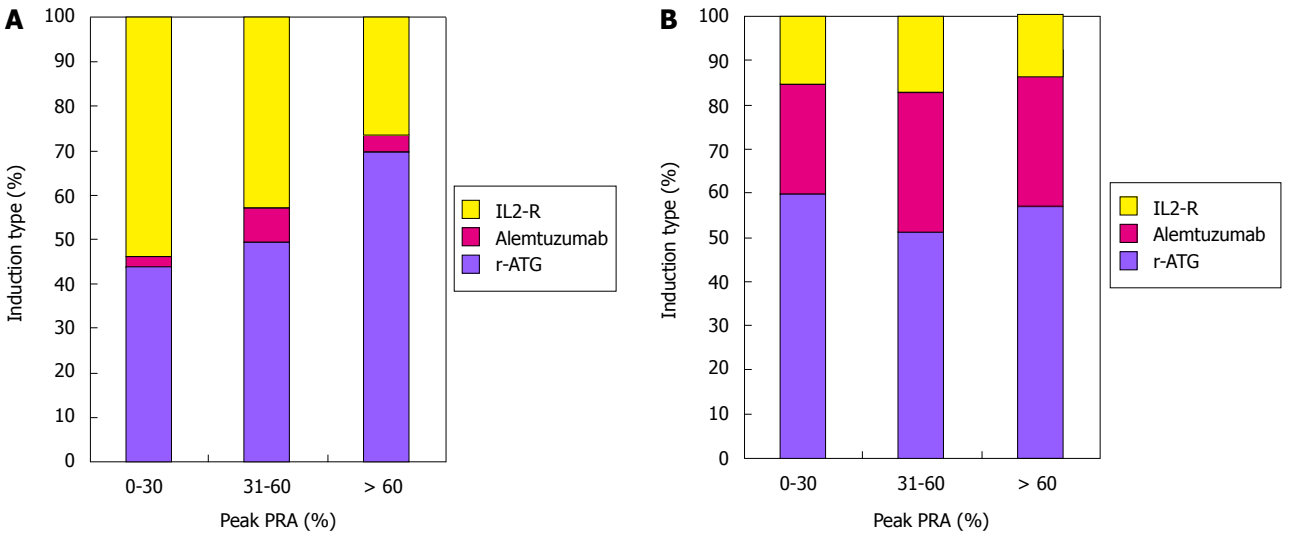

Figure 1 Trends in the use of induction agents stratified by peak-panel reactive antibody in steroid maintenance (A) and early steroid withdrawal (B) groups. Alemtuzumab is used more commonly in steroid withdrawal group. r-ATG: Rabbit- antithymocyte globulin; PRA: Panel reactive antibody.

Demographic variables for the different induction groups were collected. Graft was considered failed when one of the following occurred: need for maintenance dialysis, re-transplantation or patient death. Over all and death-censored graft as well as patient survivals were compared between ESW and CSM groups for each peak-PRA group after adjusting for pre-specified variables. We decided to use an adjusted model in the analysis due to substantial variations in the demographic features for ESW *vs* CSM in each peak-PRA category. The co-variates known to have adverse impact on the graft outcome and included in the model were donor related factors: age, gender, expanded criteria donor kidney, donation after cardiac death kidney, death from cerebrovascular accident; recipient related factors: age, African American race, diabetes mellitus, dialysis duration, number of HLA mismatches; and transplant related factors: cold ischemia time, induction type, delayed graft function (DGF, defined as the need for dialysis within the first week after transplantation), previous transplant, 12 mo acute rejection, and transplant year. Most of the patients were discharged on tacrolimus as the CNI agent; hence we did not include the type of CNI agent in the model. Since older KTRs could be more prone to the risks of enhanced immunosuppression, a further analysis was done comparing adjusted overall and death-censored graft failure risks as well as patient death risk between CSM and ESW groups in the subgroup of patient $\geqslant$ 60 years of age stratified by the peak-PRA class.

Statistical analysis

Comparisons among groups were made using 2-tailed *t*-test for continuous variables and chi square test for categorical variables. Values were expressed as mean ± SD, median with range or percentage. When there were missing data for different variables/risk factors in the registry, we assumed absence of the risk factor for the purpose of analysis. Less than 2% of the data were missing for different variables used in the analysis except for treated acute rejection where 20%-25% of data were missing. Adjusted (multivariate, after correcting for the confounding variables listed above) over all and death-censored graft as well as patient survivals were calculated and were compared between CSM *vs* ESW groups within each peak-PRA category using a Cox regression model. A further analysis comparing adjusted overall and death-censored graft failure as well as patient death risks in CSM *vs* ESW was performed in the subgroup of patients $\geqslant$ 60 years of age stratified by the peak-PRA class. Hazard ratio (HR and 95%CI) were calculated. A P value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 14.

RESULTS

Demographic characteristics

Median follow-up in months with range by peak-PRA category were as follows: 0%-30%, 36.1 (21.5 to 60.0); 31%-60%, 36.0 (20.4 to 60.7); > 60%, 35.1 (18.0 to 57.4). Trends in the utilization of different induction agents stratified by steroid use and peak-PRA class are shown in Figure 1. In CSM group, proportion of patients receiving r-ATG induction increased from low to high peak PRA groups. Alemtuzumab was predominantly used in ESW group.

A total of 42851 DDK recipients were included in the analysis. Among these patients, 9172 (21%) were in the ESW group and 33679 (79%) in CSM group. Distribution of the 42851 study patients by peak-PRA class was as follows: 0%-30%, n = 32617 (steroid = 25218, no steroid = 7399); 31%-60%, n = 4345 (steroid = 3495, no steroid = 850); > 60%, n = 5889 (steroid = 4966, no steroid = 923). There were substantial variations for steroid *vs* no steroid groups

Table 1 Demographic features

	Peak-PRA 0%-30%		Peak-PRA 31%-60%		Peak-PRA > 60%	
	Steroid (*n* = 25218)	No steroid (*n* = 7399)	Steroid (*n* = 3495)	No steroid (*n* = 850)	Steroid (*n* = 4966)	No steroid (*n* = 923)
Donor factors						
Age	38 ± 17	39 ± 17[b]	37 ± 17	38 ± 18	35 ± 15	35 ± 16
Gender (M/F) %	59/41	59/41	60/40	57/43	60/40	66/34[b]
Death from CVA (%)	42	40[a]	38	41[a]	36	35[a]
ECD kidney (%)	18	20[d]	15	22[d]	8	10
DCD kidney (%)	7.7	9.1[d]	6.1	7.6	6.7	8.1
Recipient factors						
Age (yr)	51 ± 13	53 ± 13[d]	37 ± 17	38 ± 17	47 ± 13	49 ± 13
Gender (M/F) %	66/34	67/33	51/49	51/49	37/63	34/66
African American	30	26[d]	28	28	33	31
Diabetes	33	36[d]	29	34[b]	25	28
Pre-transplant dialysis (%)	91	88[d]	89	84[d]	91	91
Dialysis duration (mo)	45 ± 34	44 ± 35[a]	49 ± 42	47 ± 42	61 ± 51	59 ± 52
Previous transplant (%)	7.3	4.6[d]	21.8	14.6[d]	44.2	37.9[d]
HLA mismatches	3.7 ± 1.8	3. 7 ± 1.9	3.5 ± 2.0	3.1 ± 2.0[a]	3.1 ± 2.0	3.0 ± 2.0
Transplant- related factors						
Cold ischemia (h)	18.1 ± 8.1	18.8 ± 8.0[d]	18.3 ± 7.9	20.8 ± 10.2[d]	18.4 ± 8.1	19.3 ± 8.2[b]
Delayed graft function (%)	24.4	19.5[d]	22.9	19.6[a]	26	20.8[b]

P value is for steroid *vs* no steroid: [a]$P < 0.05$, [b]$P < 0.01$; [d]$P < 0.001$. CVA: Cerebrovascular accident; DCD: Donation after cardiac death; ECD: Expanded criteria donor; HLA: Human leukocyte antigen; PRA: Panel reactive antibody.

Table 2 Adjusted overall and death-censored graft failure risks as well as patient death risk for chronic steroid maintenance *vs* early steroid withdrawal groups in patients ⩾ 60 years of age

	Adjusted overall graft failure risk		Adjusted death-censored graft failure risk		Adjusted patient death risk	
PRA class	HR	95%CI	HR	95%CI	HR	95%CI
0%-30%	1.28[d]	1.14-1.47	1.27[b]	1.10-1.45	1.43[d]	1.22-1.64
31%-60%	1.04	0.71-1.47	1.04	0.70-1.54	1.20	0.79-1.81
> 60%	0.74	0.51-1.09	0.71	0.48-1.09	0.76	0.49-1.19

[b]P: 0.001, *vs* overall graft survivals; [d]$P < 0.001$, *vs* death-censored graft survivals. PRA: Panel reactive antibody.

under each peak-PRA group as shown in Table 1. Of note, a consistently higher proportion of patients with previous transplants and DGF were discharged on steroid maintenance. There were more diabetics in the ESW groups likely reflective of the practice of avoiding steroids in patients with high blood sugar. Another observation is the trend in increasing dialysis duration and proportion of patients with prior transplants from the lowest to highest peak-PRA groups.

Impact of steroid use on graft survival by level of sensitization

Adjusted overall and death-censored graft survivals for CSM *vs* ESW groups stratified by peak-PRA classes are shown in Figure 2. In patients with peak-PRA 0%-30%, there was higher adjusted overall graft failure risk (HR 1.11, 95%CI: 1.03-1.20, $P = 0.009$) but similar death-censored graft failure risk (HR 1.06, 95%CI: 0.98-1.14, $P = 0.16$) for CSM *vs* ESW groups. Adjusted over all (HR 1.04, 95%CI: 0.85-1.28, $P = 0.70$) and death-censored (HR 0.97, 95%CI: 0.78-1.21, $P = 0.81$) graft failure risks were similar for CSM *vs* ESW groups in the 31%-60% peak-PRA group. For patients in the > 60% peak-PRA group, adjusted overall graft failure risk was similar (HR 0.90, 95%CI: 0.76-1.08, $P = 0.25$) but death-censored graft failure risk was lower (HR 0.84, 95%CI: 0.71-0.99, $P = 0.04$) for CSM *vs* ESW groups.

A further analysis was performed comparing adjusted overall and death-censored graft survivals between ESW and CSM groups in patients ⩾ 60 years of age stratified by peak-PRA class as shown in Table 2. CSM was associated with higher adjusted overall and death-censored graft failure risks in the 0%-30% peak-PRA group. There were no significant graft outcome differences between the groups for patients in the 31%-60% and > 60% peak-PRA groups.

Impact of steroid maintenance on patient survival by level of sensitization

Adjusted patient survivals for the different peak-PRA groups are shown in Figure 3. Adjusted patient death risks were higher for CSM *vs* ESW groups in peak-PRA groups 0%-30% (HR 1.29, 95%CI: 1.16-1.43, $P < 0.001$) and 31%-60% (HR 1.39, 95%CI: 1.03-1.87, $P = 0.03$). There was no difference in adjusted patient death risk for ESW *vs* CSM in the > 60% peak-PRA group. In KTRs ⩾ 60 years of age, adjusted patient death risk was higher for CSM *vs* ESW group in 0%-30%

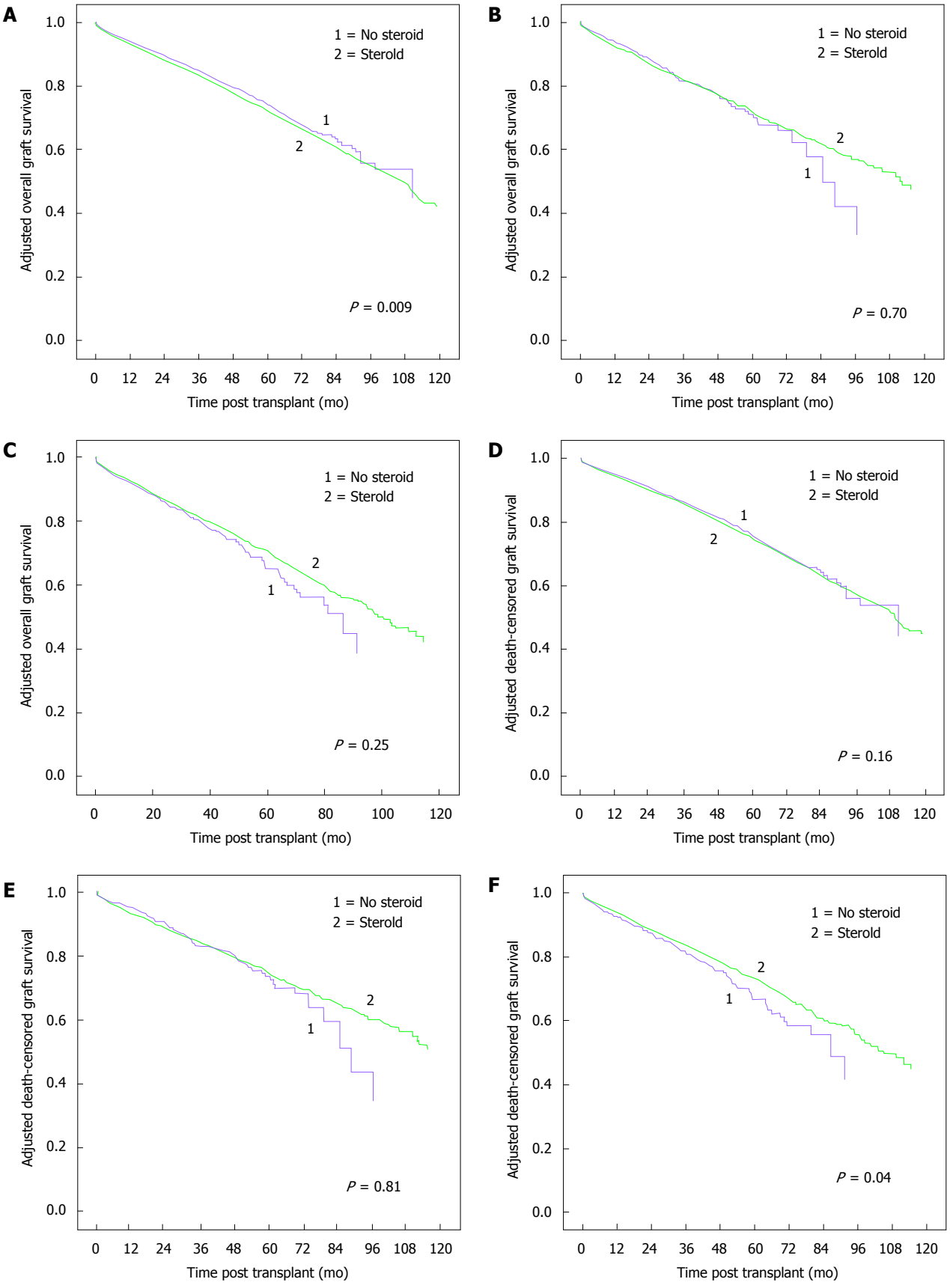

Figure 2 Over all adjusted graft (A-C) and death-censored graft (D-F) survivals in peak panel reactive antibody classes 0%-30%; 31%-60% and > 60% respectively. Note the association of steroid maintenance with decreased overall graft survival in the peak-PRA 0%-30% group and improved death-censored graft survival in peak-PRA > 60% group. PRA: Panel reactive antibody.

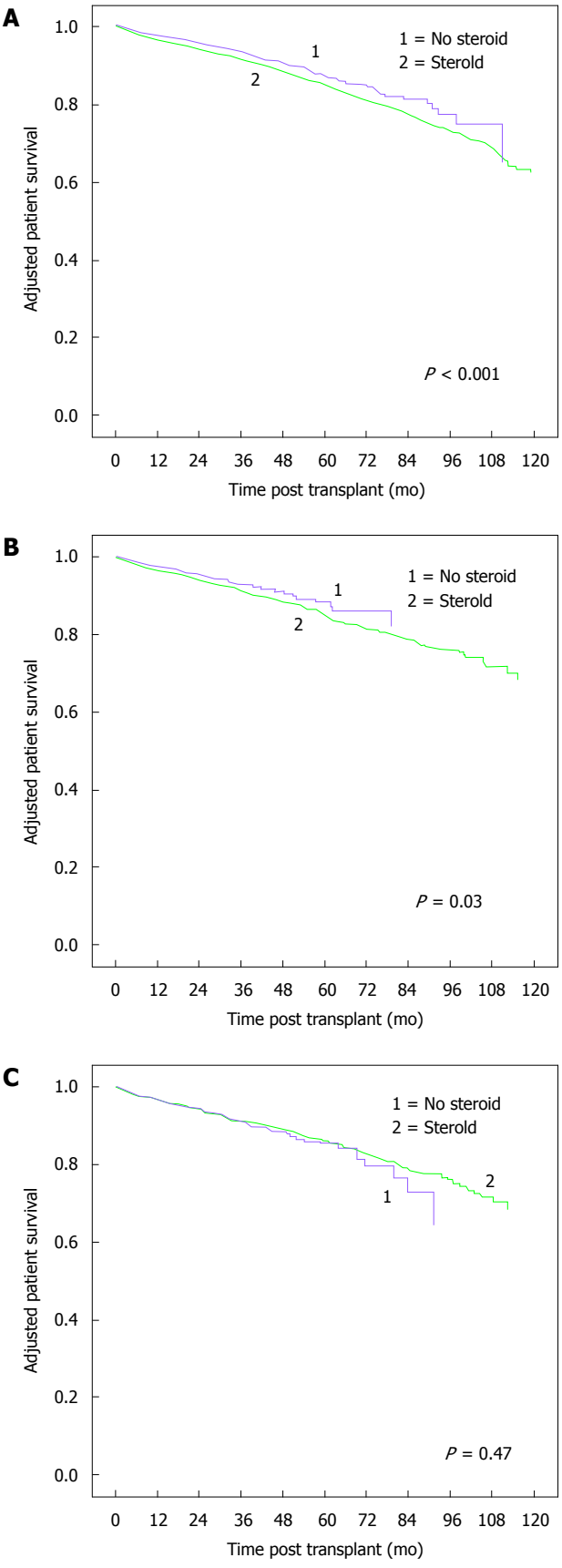

Figure 3 Adjusted patient survival (A-C) in peak-panel reactive antibody classes 0%-30%, 31%-60% and > 60% respectively. Note the inferior patient survival associated with steroid maintenance in peak-PRA groups 0%-30% and 31%-60%. PRA: Panel reactive antibody.

peak-PRA class. Adjusted patient death risks were similar between CSM and ESW groups for higher peak-PRA classes (Table 2).

DISCUSSION

Our study demonstrated an association between the addition of steroid to a CNI/MMF maintenance regimen and risk of patient death in DDK transplant recipients considered low immunological risk defined as peak-PRA 0%-30%. Increased overall but similar death-censored graft survival suggests an increased risk for death with functioning graft associated with steroid use in this group. Steroid use was associated with an improved death-censored graft survival without adversely affecting patient survival in high immune risk patients with peak-PRA > 60%. In the subgroup of patients $\geqslant$ 60 years of age, steroid use was associated with inferior graft and patient outcomes in low immune-risk patients and no significant benefits in high-immune risk patients. All study patients received perioperative induction therapy.

Several studies in the past have looked at the safety and efficacy of ESW in KTR[6-8]. Woodle *et al*[9] performed a prospective, randomized multicenter trial comparing early corticosteroid withdrawal *vs* long-term, low-dose corticosteroid therapy in KTR who received antibody induction followed by CNI/ MMF based immunosuppression therapy. Early steroid cessation was associated with slightly higher risk of steroid sensitive Banff 1A cellular rejection which did not translate into adverse long term graft survival and function. ESW was associated with reductions in the incidences of new onset diabetes after transplant, hypertriglyceridemia, and significant weight gain[9]. Risk factors for the development of acute rejection in patients who underwent ESW included repeat transplantation and lack of r-ATG use. There was a trend towards increased acute rejection in patients with PRA greater than 50%[13]. Rates of acute rejection, graft survival and patient survival were 40%, 88% and 96% respectively in a pilot study involving 25 high immune risk patients who underwent ESW and followed for 402 d[14]. Rates of acute rejection were lower in high immune risk patients who underwent steroid withdrawal if they received r-ATG induction. A recent analysis of the OPTN database involving large number of repeat KTR showed no added benefits of steroid maintenance in terms of patient or graft survival in the group that received perioperative induction with r-ATG[15]. A meta-analysis of 15 randomized control trials involving 3520 patients showed no significantly increased risk for acute rejection following very ESW if patients received perioperative induction followed by tacrolimus as part of maintenance therapy[16]. In fact, a recent study involving close to 42000 patients reported a highly significant association between maintenance steroid dose and death with functioning graft caused by

cardiovascular disease or infection beyond the first year following DDK transplantation[17]. Neither tacrolimus nor mycophenolic acid use was associated with risk for death with functioning graft.

It makes intuitive sense that the higher immunologic risk KTR might benefit from enhanced immunosuppression with chronic steroid use. To our best knowledge, no previous studies specifically evaluated to find a threshold peak-PRA level beyond which the benefits of enhanced immunosuppression with CSM in terms of improved graft outcome begin to emerge. Our analysis did not reveal any clinically detectable graft and patient survival advantages in KTRs with peak PRA $\leqslant$ 60 who underwent perioperative induction therapy followed by CNI/MMF maintenance. An improved death-censored graft survival was associated with steroid maintenance in those with PRA > 60%. In the subgroup of older KTRs $\geqslant$ 60 years of age, steroid maintenance was not associated with survival benefits regardless of the level of sensitization.

One could speculate enhanced immunosuppression with risk for infectious complications as well as adverse metabolic and cardiovascular effects as possible reasons for the observed association between steroid maintenance and increased risk for death with functioning graft in low immune risk patients. Improved death censored graft survival associated with steroid use without adversely affecting patient survival in highly sensitized group suggests that favorable immunosuppressive effect of CSM in these patients is not fully offset by any adverse consequences of CSM.

In order to perform the current analysis, we utilized a cohort of patients from 2000-2008, an era before the concept of calculated panel reactive antibody (cPRA) which was introduced in 2009. The cPRA is based on the unacceptable antigens which if present in the donor would not be acceptable for the recipient. Depending on the frequency of the unacceptable antigens in the donor population, the cPRA is computed[18]. Unlike traditional PRA, cPRA provides a meaningful estimate of transplantability for most patients, as it would preclude offers from donors who could have a positive cross-match. Hence cPRA is described as a measure that provides both consistency and accountability[19]. cPRA as a concept introduced fairly recently may offer a better predictive survival as it takes into account the virtual cross-match. The contemporary cPRA is determined using extremely sensitive solid phase assays such as Luminex® that can detect very low levels of anti-HLA antibodies that may have questionable clinical relevance as compared to the poorly sensitive cell based assays used in the past to determine traditional PRA. One could speculate that PRA from previous era may reflect a higher degree of immunological risk. Our analysis of patient cohort from the traditional PRA era shows a seemingly beneficial effect of steroid maintenance only in younger patients with peak-PRA > 60%. This observation may be even more relevant to contemporary transplant recipients whose immunological risk is stratified by cPRA.

Our study has limitations. Retrospective analyses can only show associations but not causation. Despite using a multivariate model, confounding bias may still exist. Peak-PRA reflects the level of sensitization at a time point and does not give the actual degree of sensitization in the post-transplant period. Donor specific antibody (DSA) is increasingly available in current day practice which could be a more accurate determinant of the alloreactivity to specific donor and the risk of rejection. We did not have data on DSA in our study cohort. Changes in maintenance immunosuppression made after the initial hospital discharge were not captured. Hence patients who were withdrawn from steroids after hospital discharge, or if patients were initiated on steroids due to an event such as acute rejection episode at a later date would be misclassified. The impact of these misclassifications on the results likely is minimal since the non-differential nature of such influence tends to deflate results toward the null[20]. A recent registry analysis identified African American race, re-transplants, highly sensitized recipients, recipients with Medicaid, elevated HLA mismatches and older donor age as risk factors for new initiation of steroids in DDK recipients who were initially discharged on an ESW regimen[21]. There were differences in the patterns of induction therapy used in ESW *vs* CSM groups under each peak-PRA category. We attempted to minimize the impact of this by including type of induction therapy as a variable in the multivariate model. Therapeutic levels of CNI and doses of MMF were not available that could potentially influence graft outcomes. Possibility of type 1 error cannot be excluded. Despite these limitations, relatively large number of study patients from a national cohort adds to the validity of our findings.

In summary, our analysis showed that steroid can be safely withdrawn early and potentially could even be beneficial in sensitized DDK transplant recipients with a peak PRA $\leqslant$ 60% as well as elderly patients regardless of their degree of HLA sensitization provided these patients receive perioperative induction therapy followed by CNI (in particular tacrolimus)/MMF maintenance. On the other hand, steroid maintenance appears beneficial in the subgroup of highly sensitized younger patients. Randomized trials with sufficient size and follow up will be needed to further evaluate these clinically important findings.

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organizations imply endorsement by the United States Government.

COMMENTS

Background

Analysis of the beneficial effects of steroid maintenance in kidney transplant recipients (KTR) stratified by level of sensitization.

Innovations and breakthroughs

Beneficial effects of steroid maintenance were observed only in highly sensitized younger KTR with peak-panel reactive antibody > 60%.

Applications

In clinical transplantation.

Peer-review

The present study evaluated the probable threshold levels of sensitization at which there a benefit with maintenance of steroids in deceased-donor KTR. The study is very well written, the results are clearly presented and the discussion and limitations of the study adequately addressed.

REFERENCES

1 KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

2 **Vincenti F**, Monaco A, Grinyo J, Kinkhabwala M, Roza A. Multicenter randomized prospective trial of steroid withdrawal in renal transplant recipients receiving basiliximab, cyclosporine microemulsion and mycophenolate mofetil. *Am J Transplant* 2003; **3**: 306-311 [PMID: 12614286]

3 **Matas AJ**, Kandaswamy R, Gillingham KJ, McHugh L, Ibrahim H, Kasiske B, Humar A. Prednisone-free maintenance immunosuppression-a 5-year experience. *Am J Transplant* 2005; **5**: 2473-2478 [PMID: 16162197]

4 **Rostaing L**, Cantarovich D, Mourad G, Budde K, Rigotti P, Mariat C, Margreiter R, Capdevilla L, Lang P, Vialtel P, Ortuño-Mirete J, Charpentier B, Legendre C, Sanchez-Plumed J, Oppenheimer F, Kessler M. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807-814 [PMID: 15818323]

5 **Kumar MS**, Heifets M, Moritz MJ, Saeed MI, Khan SM, Fyfe B, Sustento-Riodeca N, Daniel JN, Kumar A. Safety and efficacy of steroid withdrawal two days after kidney transplantation: analysis of results at three years. *Transplantation* 2006; **81**: 832-839 [PMID: 16570004]

6 **Vincenti F**, Schena FP, Paraskevas S, Hauser IA, Walker RG, Grinyo J. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. *Am J Transplant* 2008; **8**: 307-316 [PMID: 18211506]

7 **Pascual J**, Zamora J, Galeano C, Royuela A, Quereda C. Steroid avoidance or withdrawal for kidney transplant recipients. *Cochrane Database Syst Rev* 2009; **1**: CD005632 [PMID: 19160257 DOI: 10.1002/14651858.CD005632.pub2]

8 **Schiff J**, Cole EH. Renal transplantation with early steroid withdrawal. *Pediatr Nephrol* 2009; **24**: 243-251 [PMID: 18535842 DOI: 10.1007/s00467-008-0876-0]

9 **Woodle ES**, First MR, Pirsch J, Shihab F, Gaber AO, Van Veldhuisen P. A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early (7 day) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. *Ann Surg* 2008; **248**: 564-577 [PMID: 18936569 DOI: 10.1097/SLA.0b013e318187d1da]

10 **Knight SR**, Morris PJ. Steroid avoidance or withdrawal after renal transplantation increases the risk of acute rejection but decreases cardiovascular risk. A meta-analysis. *Transplantation* 2010; **89**: 1-14 [PMID: 20061913 DOI: 10.1097/TP.0b013e3181c518cc]

11 **Rizzari MD**, Suszynski TM, Gillingham KJ, Dunn TB, Ibrahim HN, Payne WD, Chinnakotla S, Finger EB, Sutherland DE, Kandaswamy R, Najarian JS, Pruett TL, Kukla A, Spong R, Matas AJ. Ten-year outcome after rapid discontinuation of prednisone in adult primary kidney transplantation. *Clin J Am Soc Nephrol* 2012; **7**: 494-503 [PMID: 22282482 DOI: 10.2215/CJN.08630811]

12 **Luan FL**, Steffick DE, Gadegbeku C, Norman SP, Wolfe R, Ojo AO. Graft and patient survival in kidney transplant recipients selected for de novo steroid-free maintenance immunosuppression. *Am J Transplant* 2009; **9**: 160-168 [PMID: 18976304 DOI: 10.1111/j.1600-6143.2008.02442.x]

13 **Woodle ES**, Alloway RR, Hanaway MJ, Buell JF, Thomas M, Roy-Chaudhury P, Trofe J. Early corticosteroid withdrawal under modern immunosuppression in renal transplantation: multivariate analysis of risk factors for acute rejection. *Transplant Proc* 2005; **37**: 798-799 [PMID: 15848535]

14 **Alloway RR**, Hanaway MJ, Trofe J, Boardman R, Rogers CC, Hanaway MJ, Buell JF, Munda R, Alexander JW, Thomas MJ, Roy-Chaudhury P, Cardi M, Woodle ES. A prospective, pilot study of early corticosteroid cessation in high-immunologic-risk patients: the Cincinnati experience. *Transplant Proc* 2005; **37**: 802-803 [PMID: 15848537]

15 **Sureshkumar KK**, Hussain SM, Nashar K, Marcus RJ. Steroid maintenance in repeat kidney transplantation: influence of induction agents on outcomes. *Saudi J Kidney Dis Transpl* 2014; **25**: 741-749 [PMID: 24969182]

16 **Zhang X**, Huang H, Han S, Fu S, Wang L. Is it safe to withdraw steroids within seven days of renal transplantation? *Clin Transplant* 2013; **27**: 1-8 [PMID: 23072524 DOI: 10.1111/ctr.12015]

17 **Opelz G**, Döhler B. Association between steroid dosage and death with a functioning graft after kidney transplantation. *Am J Transplant* 2013; **13**: 2096-2105 [PMID: 23750878 DOI: 10.1111/ajt.12313]

18 **Nikaein A**, Cherikh W, Nelson K, Baker T, Leffell S, Bow L, Crowe D, Connick K, Head MA, Kamoun M, Kimball P, Klohe E, Noreen H, Rebellato L, Sell T, Sullivan K, Land G. Organ procurement and transplantation network/united network for organ sharing histocompatibility committee collaborative study to evaluate prediction of crossmatch results in highly sensitized patients. *Transplantation* 2009; **87**: 557-562 [PMID: 19307794 DOI: 10.1097/TP.0b013e3181943c76]

19 **Cecka JM**. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. *Am J Transplant* 2010; **10**: 26-29 [PMID: 19958328 DOI: 10.1111/j.1600-6143.2009.02927.x]

20 **Copeland KT**, Checkoway H, McMichael AJ, Holbrook RH. Bias due to misclassification in the estimation of relative risk. *Am J Epidemiol* 1977; **105**: 488-495 [PMID: 871121]

21 **Schold JD**, Santos A, Rehman S, Magliocca J, Meier-Kriesche HU. The success of continued steroid avoidance after kidney transplantation in the US. *Am J Transplant* 2009; **9**: 2768-2776 [PMID: 19845594 DOI: 10.1111/j.1600-6143.2009.02838.x]

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ORIGINAL ARTICLE

Retrospective Study

Effectiveness of repeated transplantations of hematopoietic stem cells in spinal cord injury

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Author contributions: Both authors contributed equally to this work.

Institutional review board statement: The study was reviewed and approved by Ethics Committee Russian State Medical University (Moscow, Russia). Since 2005 the method was approved for clinical practice.

Informed consent statement: All study participants, or their legal guardian, provided an informed written consent prior to study involvement.

Conflict-of-interest statement: Professor Andrey S Bryukhovetskiy PhD, MD, is an employee of the Federal Research Center for Specialized Types of Medical Assistance and Medical Technologies of FMBA of Russia. Professor Andrey S Bryukhovetskiy PhD, MD, owns stocks and shares in the NeuroVita Clinic of Restorative and Interventional Neurology and Therapy. Professor Andrey S Bryukhovetskiy PhD, MD, owns patent Preparation of Autologous Hematopoietic Stem Cells, Method of Production, Cryopreservation and Application for Treatment of Traumatic Diseases of Central Nervous System, Patent of Russian Federation RU No. 2283119 C1 dated 10.09.2006; International Application No. PCT/EP 2005108721 filed on 29.03.2005 Preparation of autologous stem cells, the methods of production, cryopreservation and use for therapy of traumatic diseases of central nervous system.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at neurovitaclinic@gmail.com. Participants gave written informed consent for data sharing, and the data are anonymized.

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Abstract

AIM: To evaluate the short and long-term effects of the complex cell therapy of 202 cases of spinal cord injury (SCI).

METHODS: The main arm included 202 cases of SCI and the control arm included 20 SCI cases. For the therapy the hematopoietic stem cells (HSCs) and progenitor cells (PCs) were mobilized to peripheral blood by 8 subcutaneous injections of granulocyte colony-stimulating factor (G-CSF) for 4 d and are harvested at day 5. The cells were administered to the main arm intrathecally every 3 mo for a long term (3-5 years) according to the internal research protocol international medical institute of tissue engineering. Magnetic resonance imaging of the site of injury and urodyna-

mic tests were performed every 6 mo. Motor evoked potentials (MEP), somatosensory evoked potentials (SSEP) were evaluated every 3 mo. The patients were evaluated with american spianl injury association (ASIA) index, functional independence measure index, the Medical Research Council Scale, the International Standards for Neurological Classification of Spinal Cord Injury (ISCSCI-92) and specifically developed scales. The function of bladder was evaluated by a specifically developed clinical scale. The long-term clinical outcomes were assessed for the SCI patients who received no less than 20 intrathecal transplantations of HSCs and hematopoietic precursors (HPs).

RESULTS: The restoration of neurologic deficit after HSCs and HPs transplantations was proved stable and evident in 57.4% of the cases. In 42.6% cases no neurologic improvement has been observed. In 50% of the cases the motor restoration began after the first transplantation, which is confirmed in average by 9.9 points improvement in neurologic impairment as compared to the baseline ($P < 0.05$). Repair of the urinary system was observed in 47.7% of the cases. The sensitivity improved from baseline 124.3 points to 138.4 after the first and to 153.5 points after the second transplantations of HSCs and HPs ($P < 0.05$, between the stages of research). The evaluation with ASIA index demonstrated regress of neurologic symptoms in 23 cases. Motor progress was also assessed with the ISCISCI-92 motor and sensory scores, and the data coincided with those received with the specifically developed scale. The number of the patients with the signs of locomotive repair was 56.9%. No life threatening complications or adverse effects have been observed.

CONCLUSION: The method is safe, effective and considerably improves the life quality of SCI patients. The therapy is approved for clinical use as the treatment of choice.

Key words: Spinal cord injury; Paraplegia; Tetraplegia; Hematopoietic stem cells; Stem cells; Cell therapy

Core tip: The work summarizes the 12 year experience of stem cell therapy for chronic spinal cord injury. The unique preparation of autologous hematopoietic stem cells and hematopoietic precursors was multiply administered to 202 patients. The article analyzes short and long-term benefits, short and long-term complications and the instruments that were used for their evaluation.

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INTRODUCTION

A global incidence rate of traumatic spinal cord injury (SCI) is estimated as 23 cases per million[1]. Regional incidence rates vary from 15 (Australia) to 40 (United States) cases per 1 million of population[1]. The average age at injury increased from 28.7 years in the 1970s to 42.6 years since 2010[2], still, the incidence of traumatic SCI peaks in young people[1,3].

Although spinal fractures constitute only 0.44% of all injury types, the percentage of spinal traumas has dramatically increased (over 200-fold) for the past 7 decades. The analysis predicted 800 of new SCI per 10 million of population.

For the past two decades the therapeutic advances hold a lot of promise for the patients with SCI, but none of the available therapies led to restoration of the morphological structure of spinal cord and its functions. Various therapeutic programs improve outcomes and life quality of the injured only in a few cases, but still they remain unable to repair severe neurologic deficit and restore lost functions. Surgical approaches to repair SCI are aimed at orthopedic restoration of vertebral canal anatomy, and their results remain controversial. To date, an SCI is a final verdict that entails impossibility to return to the previous way of life, to restore previous working capacity and reproductive functions, resulting in tremendous social and economic losses. The total direct costs of SCI in the United States alone are estimated at about 7.7 billion United States dollar[4].

Inefficiency of the available SCI therapies was used to be explained by the absence of regeneration potential of adult neurons, and the opportunity to restore damaged neural cells has only recently been proved[5]. By now, the first steps to develop new neurorestorational therapy of SCI have been made[6,7], although no universally acknowledged methods to restore the spinal cord after the injury are observed. Novel cell techniques and tissue engineering methods can provide the solution; so, according to the Stem Cell Summit (2009) data, 34 million of patients received transplantations of stem cells of various origin, and 1 million of them were SCI patients[8]. However, outcomes and long-term consequences of such transplantations remain as yet unknown.

The available experience is minimally documented and rather obscure, due to insufficient theoretical and experimental evidence of cell technologies, as well as underdeveloped methods of their application, when the fate of transplanted cells, their further differentiation and transformation are unclear. The crucial question of cancer development, triggered by the transplantation of stem cells, also remains unanswered. The myths and fears of possible negative consequences of stem cell therapy significantly interfere with the research and progress in the area.

Table 1 Sex, age, level of injury distribution of patients with traumatic disease of spinal cord (main group)

No. of patients	202 (1008 case histories)
Age	From 19 to 51 yr
Gender	Males - 156, females - 46
Years post injury	Less than 1 yr - 11 From 1 to 5 yr - 144 Over 5 yr - 47
Level of spinal cord injury	Cervical level - 93 Thoracic level - 98 Lumbar level - 11
Type of injury	Complete - 43 Incomplete - 159
No. of transplantations	No less than 20 HSCs and HPs transplantations
Average number of transplanted cells	5.8×10^6

HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors.

We have transplanted cells for SCI for 25 years both in research and in clinical practice and have accumulated substantial experience of victories and defeats administrating allogeneic and xenogeneic fetal neural and mesenchymal cells, isolated from animal and human embryos of 10-24 gestation weeks, as well as embryonic stem neural cells, obtained from human blastocyst. This experience is summed up in our book[9], and to date, we have refused from the clinical application of allogeneic and xenogeneic cell material for SCI. We believe the future of the SCI therapy to belong to the suspensions, prepared from autologous stem and progenitor cells (PCs), as under the SCI condition the organism specifies and individually tailors the cells for the treatment of their own SCI, along with the advantage of null immunologic and transplantation side effects and absence of undesirable paramedical ethic, legal and religious aspects[10]. The only option to use the allogeneic stem cells for SCI is haploidentical stem cells or those of close relatives, and only after the human leukocyte antigen typing.

In the present article we would like to determine the basic parameters for the beginning of the cell therapy for SCI and the criteria to terminate it in clinical practice.

MATERIALS AND METHODS

The 12 year trial was performed under the branch program of the Russian Academy of Medical Sciences New Cell Techniques to Medicine, with the approval and under the supervision of the Scientific Board and Ethics Committee of the Russian State Medical University (Moscow, Russia). The trial was launched 2002 and was not registered in the international database for their absence. It is an open parallel controlled trial (phase Ⅰ/Ⅱ) that followed IMITE protocol (Switzerland). The trial included 202 SCI patients (1008 case histories) that made trial group 1, see Table 1. According to the protocol, we evaluated the control group that included 20 SCI patients matched by age, sex and level of injury, see Table 2. The enrolled patients signed the Informed Consent. Trial participants met the following eligibility criteria: SCI occurred at least 12 mo prior to the inclusion into the trial; age between 15 and 60; adequate end organ function; adequate bone marrow function, negative pregnancy test; written, voluntary, informed consent. Exclusion criteria were acute infections, severe hematologic disorders; contraindications for MRI, pregnancy or breast feeding, grade Ⅲ/Ⅳ cardiac problems as defined by the New York Heart Association Criteria; severe and/or uncontrolled medical diseases; known diagnosis of human immunodeficiency virus (HIV) infection; previous radiotherapy to ⩾ 25% of the bone marrow; major surgery within 6 wk prior to study entry; known malignant tumours. All patients received conventional pharmaceutical treatment and intensive rehabilitation: exercise therapy, physiotherapy and massage. The suspension of HSCs and hematopoietic precursors (HPs) was intrathecally administered to the patients of the main arm every three months for 3-5 years. To produce HSCs and HPs suspension the stem cells (SC) and PCs are mobilized to peripheral blood by 8 subcutaneous injections of granulocyte-colony stimulating factor (G-CSF) every 10-12 h for 4 d. First three days the G-CSF dose is 2.5 μg per kilogram of body weight, the last day the dose is doubled. The stem cells and precursors are harvested at day 5 in blood cell separator (COBE-spectra, Gambro BCT, United States), using a disposable system for separation and standard solutions. The separation lasts 3-4 h, depending on the speed of the procedure, weight of the patient and blood test results. The red blood cells are removed from the obtained material in a conventional way, and the received leukoconcentrate is examined. On average, the volume of the material varies from 300 to 400 mL. The material is evaluated according to total number of nuclear cells (NCs) in the sediment and according to $CD34^+$ cells per a kilogram of the patient's weight. The NCs in the sediment are determined by counting in Gorjaev's chamber. The percentage of $CD34^+$ is determined by flow cytometry method by FACScan (Becton Dickinson, United States). Previously we have provided a detailed analysis of the preparation[10].

Table 2 Sex, age, level of injury distribution of patients with traumatic disease of spinal cord (control group)

No. of patients	20 (62 case histories)
Age	From 18 to 44 yr
Gender	Males - 13, females - 7
Years post injury	< 1 yr - 6 From 1 to 5 yr - 10 Over 5 yr - 4
Level of spinal cord injury	Cervical level - 14 Thoracic level - 4 Lumbar level - 2
Type	Complete - 12 Incomplete - 8

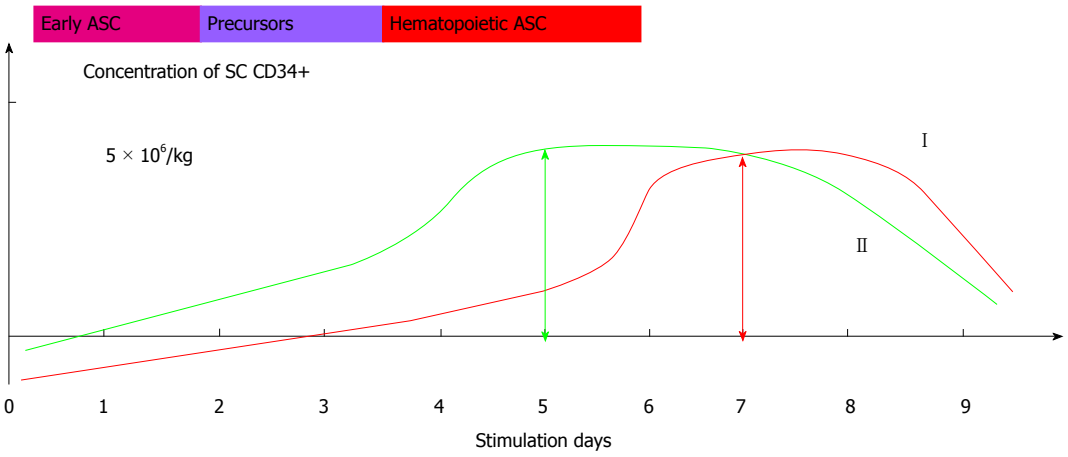


Figure 1 Obtaining cell preparations in various modes of granulocyte colony-stimulating factor stimulation.

Table 3 The characteristics and basic differences of the hematopoietic stem cells and hematopoietic precursors preparation from the preparation of hematopoietic stem cells used for bone marrow transplantation

Technique	G-CSF dose	Period of administration (d)	Stimulation regimen	Cell markers	Cryopreservant
Administration of HSCs in blood	10-20 μg/kg	6-7	1 in 24 h	CD34+, CD45+ HLA DR+, CD38+ Gp130±	10%-20% DMSO
Administration of HSC and HPs in CSF	5 μg/kg; double dose at day 4	5	2 in 24 h	CD34+, CD45- HLA DR±, CD38± Gp130+	5%-10% DMSO + polyglucin

HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors; G-CSF: Granulocyte-colony stimulating factor.

The standardized and certified HSCs and HPs were uniformly dispensed in 20 tubes and cryopreserved by adding dimethyl sulfoxide in 5% final concentration, frozen down at a rate of 1 ℃/min up to a temperature point of -80 ℃ or -120 ℃ in a programmed freezer and further stored in liquid nitrogen or liquid nitrogen vapor. The cell material is characterized in Figure 1 and Table 3. Before administration the cells are thawed in +37 ℃ water bath and washed by double centrifugation with 0.9% NaCl. According to $CD34^+$ count, an average dose of the cells is 5.8×10^6 in a tube. The main trial group received intrathecal administrations (no less than 20) of the HSCs and HPs suspension. The autologous HSCs and HPs were harvested once in 101 patients (50%), twice in 68 patients (33.7%), and three times in 33 patients (16.3%). Totally, during the whole period of observation, the patients received 1790 intrathecal transplantations of autologous HSCs and HPs. The control group patients received analogous treatment, excluding intrathecal administration of HSCs and HPs.

The patients were clinically and paraclinically evaluated according to the protocol. Evaluation of neurologic condition included tests for locomotion and sensation, bladder and bowel functions, level of injury and its completeness/incompleteness. Safety evaluation was based on the frequency of adverse events, particularly adverse events leading to discontinuation of treatment and on the number of abnormal laboratory values.

Neurological response was assessed every 3 mo, by an examination performed by a neurologist and recorded according to american spianl injury association (ASIA) scale and functional independence measure (FIM) scale. Changes from baseline in neurological status grades and body weight were summarised at defined intervals and produced in the tables of summary statistics.

MRI scan of the CNS and urodynamic tests were performed every 6 mo. Motor evoked potentials (MEP), Somatosensory evoked potentials (SSEP) examinations were performed every 3 mo. Urodynamic tests were performed every 6 mo. To evaluate motor activity we used specifically developed scale of clinical restoration of motor function[9,10] that estimated muscle force in the extremities, range of active movement and movement pace, to calculate the total score of motor activity. Additionally, motor restoration was evaluated with the Medical Research Council Scale that estimates (from 0 to 5 points, depending on the degree of manifestation) the range of active and passive movements, as well as the strength of a body and extremities. Sensitive disorders were evaluated with specifically developed scale of sensation restoration[10] that included 2-point testing of pain, temperature and deep sensation on dermatome on each side, and evaluation of the feeling of "heaviness" in resting muscles and after training in the lower and upper extremities, abdomen and back.

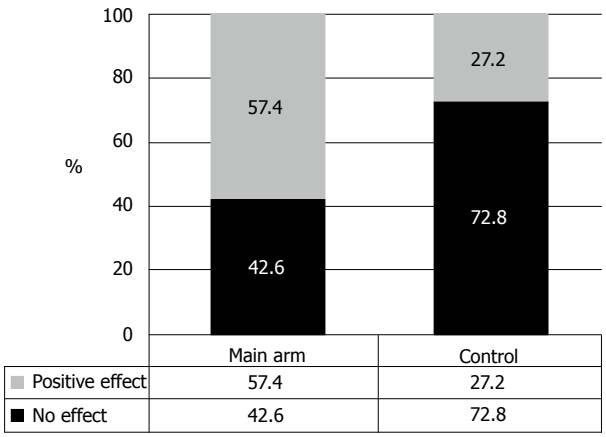


Figure 2 General effectiveness of restoration of the spinal functions after hematopoietic stem cells and hematopoietic precursors transplantation.

Completeness/incompleteness of SCI was assessed according to neurologic symptoms: lower paraplegia, conduction anesthesia and urine retention. Minimal movements or hypoesthesia below the level of injury were evaluated as an incomplete injury (no injury equals 0, an incomplete functional injury of spinal cord equals 1, a complete functional injury of spinal cord is 2).

The function of bladder was evaluated by specifically developed clinical scale to estimate the restoration of bladder function that included 3-point assessment of urination feeling and 5-point assessment of urine retention[10]. The total score, denoting absence of neurologic bladder disorders, equals 8 points. All patients passed complex urodynamic tests. Besides, the effectiveness of the intrathecal transplantation of HSCs and HPs in chronic SCI was evaluated with ASIA index, FIM index and the International Standards for Neurological Classification of Spinal Cord Injury (ISCSCI-92).

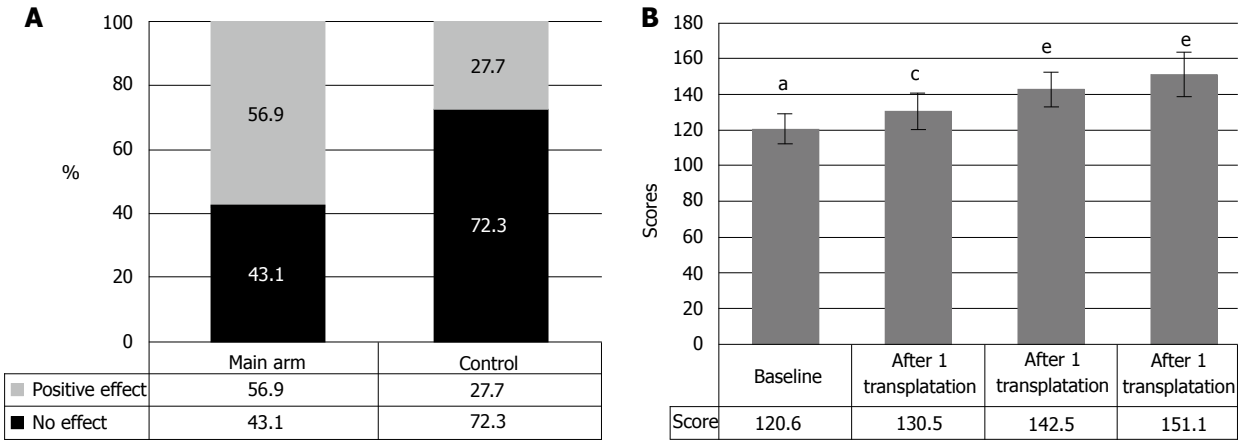
The main criteria of effectiveness were improvement of neurologic symptoms (motor, sensitive and bladder and bowel function). The expectation period for the improvement to manifest was individual in every case, depending on the scope of injury, years post injury and functional impairment. The results of the therapy manifested from 1-3 d to 24-36 mo post transplantation and were evaluated by the clinical indexes of ASIA and FIM. Patients were considered in response if at least one of the following criteria were met: (1) An unequivocal improvement of SSEP, MEP; (2) An unequivocal sign of tissue regeneration at MRI; (3) An unequivocal improvement of UT; and (4) Changes from baseline in neurological status grades (ASIA, FIM).

The statistical review of the study was performed by the biomedical statistician of the School of Biomedicine, Far Eastern Federal University. The material was statistically processed with SPSS 13 software. Statistical significance of the data was evaluated with Student' s coefficient, and analysis of variance analysis of variance and χ^2 method. The data were considered statistically significant at $P < 0.05$.

RESULTS

General efficacy of the intrathecal transplantation of HSCs and HPs

Clinical efficacy was evaluated after three years of therapy by standard neurologic examination and registration of the results in specifically developed forms. The analysis of the registered data demonstrated efficacy of the intrathecal transplantation of HSCs and HPs in 57.4% of the patients, concerning motor and sensitive restoration, as well as repair of bowel and bladder functions (Figure 2). As it can be seen from Figure 2, we observed no neurologic improvement in 42.6% cases, which can be explained by underdeveloped inclusion/exclusion criteria. To date, it is clear that the method demands rigorous screening of the patients for this therapy that will further entail the development of clearer indications and contraindications for the intrathecal transplantation of HSCs and HPs. The size of lesion, its location, type and anatomic continuity of bone structures were of prior importance in this therapy. The analysis of ineffective cases of HSCs and HPs transplantation showed that in major part of the cases (25.2%) the size of spinal cord (SC) lesion exceeded 50% of the spinal cord cross-wise and one segment long-wise, according to MRI. Other reason for the inefficacy of the intrathecal transplantation of HSCs and HPs seems to be the unnoticed moderate or slight disorder of CSF circulation, associated with CSF hypertension, instability of the spinal segment in the injury site and/or scars and cicatrices of the spinal cord that hinder the circulation of CSF. Refusal of the patients from rehabilitative therapy (40.6% of cases) has also significantly contributed to the inefficacy of the therapy. The patients considered administered transplantations sufficient for the recovery and neglected the rehabilitation. In 10.6% cases, the patients negated positive results of the therapy, although the medical exercise instructors and attending doctors observed neurologic progress. Only video records that were taken in the beginning of the treatment and in the course of it, served a decisive argument to confirm functional repair. The therapy that took from 5 to 8 years showed that these patients demonstrated good clinical results of SC functions' repair. However, this trial included only the patients who received no less than 20 transplantations of HSCs and HPs. In other cases (8.2%) the reason of inefficacy remained unclear, prompting necessity of further research. Moreover, we did not find correlation between the number of transplanted HSCs and HPs and transplantation efficacy [$P = 0.1$ ($P > 0.1$)], which was also confirmed by the absence of difference between the number of the transplanted cells to the patients with no effect and those with positive effect, resulting from HSCs and HPs transplantation ($5.3 \pm 0.9 \times 10^6$, as compared to $106.4 \pm 0.9 \times 10^6$, $P > 0.1$, respectively). The hypothesis that the process of repair after intrathecal administration of HSCs and PCS depends on



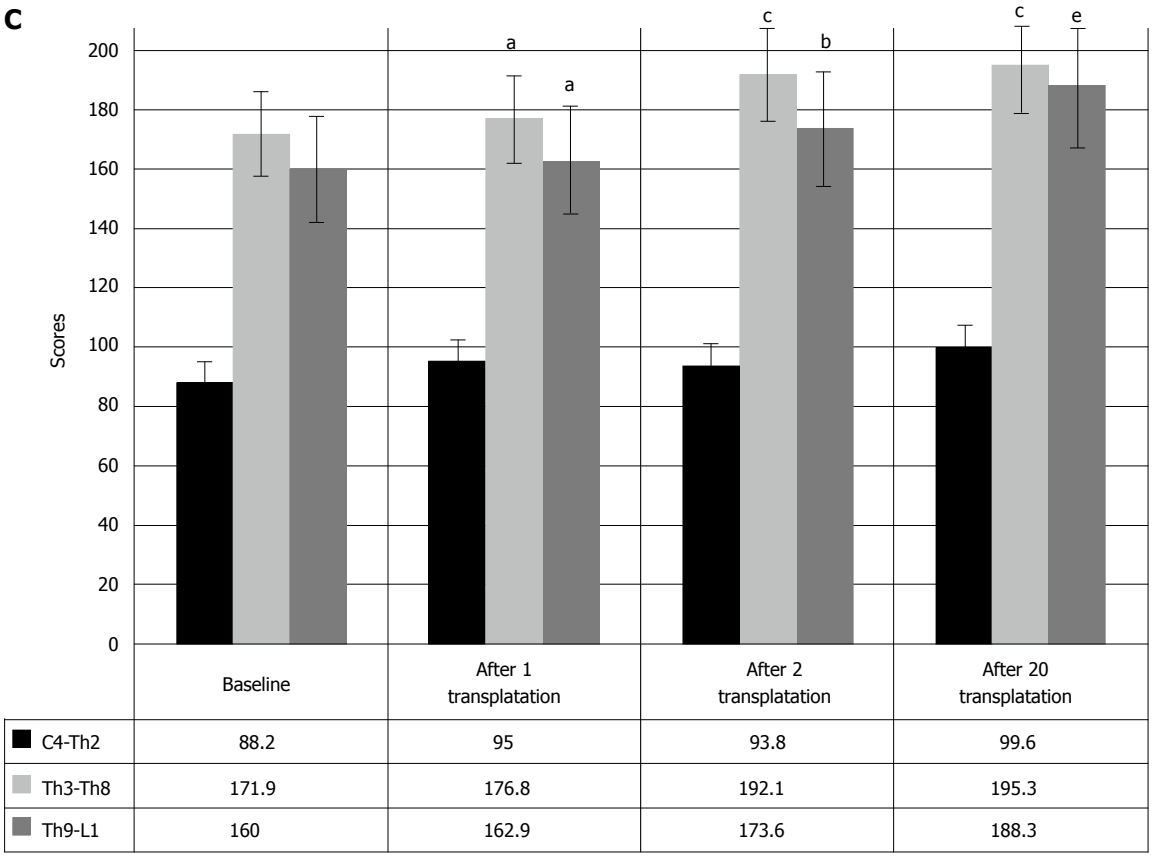
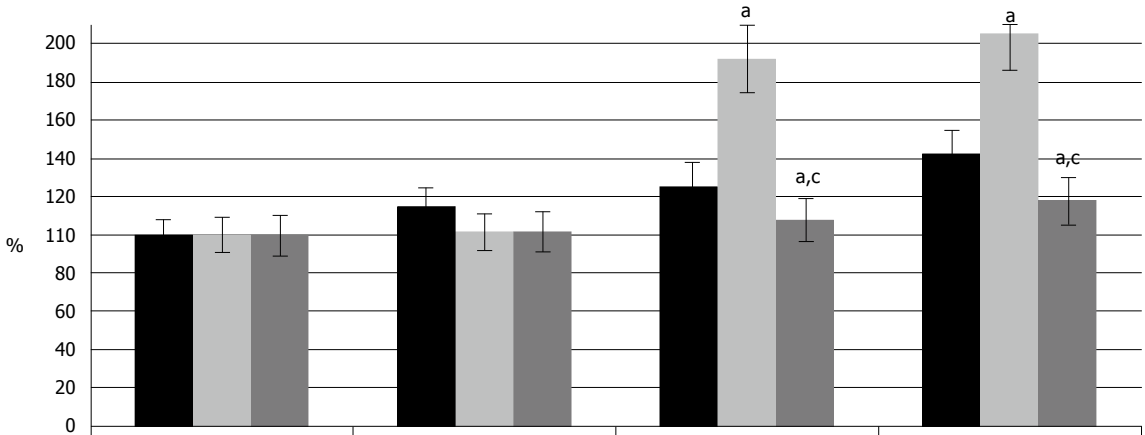


Figure 3 General effectiveness (A), clinical progress (B) and clinical picture (C). A: General effectiveness of motor functions' restoration after HSCs and HPs transplantation; B: Clinical progress in motor functions after HSCs and HPs transplantation; C: Clinical picture of motor functions after HSCs and HPs transplantation in SCI patients with different levels of injury. $^{a}P < 0.05$ as *vs* the baseline; $^{c}P < 0.05$ as *vs* the score after 1 HSCs and HPs transplantation; $^{e}P < 0.05$ as *vs* the score after 2 HSCs and HPs transplantations; $^{b}P < 0.1$ as *vs* the baseline. HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors; SCI: Spinal cord injury.

the amount of the cells ($5.3 \pm 0.9 \times 10^6$ as compared to $106.4 \pm 0.9 \times 10^6$) was not confirmed at a 90% significance level.

Evaluation of motor function repair: The efficacy of the intrathecal transplantation of HSCs and HPs was evaluated with the help of the assessment of neurologic condition that included 5-point test of muscle strength, active movements and pace of movements of the extremities on both sides. Total score for no neurologic disorder is 300 points. As seen from Figure 3A, 56.9% of the cases demonstrated improvement of neurologic symptoms, accompanied by muscle strength and muscle tone build-up, visual contractions of some groups of muscles, frequently unilateral, and further development of movements in lightweight positions. Largely, the active movements appeared 12-18 mo later during exercises on press machines. Accordingly, in 50% of the patients the motor restoration began after the first HSCs and HPs transplantation, which is confirmed in average by 9.9 points improvement in neurologic impairment as compared to the baseline



	Baseline	After 1 transplatation	After 2 transplatation	After 20 transplatation
C4-Th2	100	115.2	125	143
Th3-Th8	100	101.6	192.1	205.3
Th9-L1	100	101.9	107.7	117.6

Figure 4 Comparison of clinical motor restoration after hematopoietic stem cells and hematopoietic precursors transplantation among spinal cord injury patient with different injury levels. [a]*P* < 0.05 as *vs* the level C4-Th2; [c]*P* < 0.05 as *vs* the level Th3-Th8.

(*P* < 0.05) (Figure 3B). Repeated HSCs and HPs transplantations further enhanced neurologic improvement, that made 142.5 ± 9.7 points (*P* < 0.05, as compared to baseline and first HSCs and HPs transplantation results). Usually, intensive exercise led to strengthening of extremities' muscles, increase of range and pace of the movements, stabilization of the knee joints, ability to stand independently in the knee supporting position and development of the elements of walking with assisting devices (walkers). It should be noted that 91.2% reported no restoration of motor functions for several years, and development of the first controllable movements was extremely important for the patients and served an incentive for further training. However, the improvement of the muscle strength was often admitted by the patient no earlier than in 6-12 mo and became objective reality by the end of the second or even third year. By the sixth year, the patients are deeply convinced in the effectiveness and practicability of the therapy.

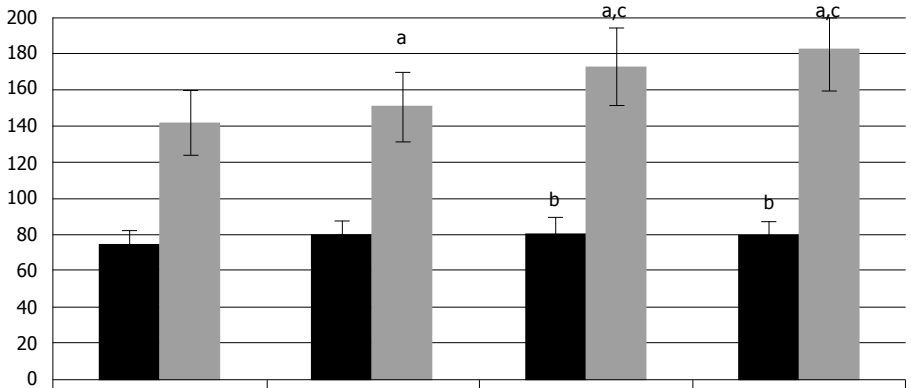
As our research demonstrated, the intrathecal transplantations of HSCs and HPs led to gradual recovery of the lost movements in chronic SCI patients, only being accompanied by specific rehabilitation. Still, rehabilitation without HSCs and HPs transplantation before enrollment into the program produced only limited effect.

Post HSCs and HPs transplantation changes of motor activity depending on the level of injury

The motor improvement was mostly observed at Th3-Th8 level of injury, specifically in 81.3% of the cases (Figure 3C). Meanwhile, cervical and lumbar SCI cases showed lesser benefit from the therapy, and functional restoration was less illustrative (Figure 4). However, the level Th3-Th8 cases demonstrated considerable repair.

Due to baseline diversity, the comparison of the clinical data between the levels of injury was done in per cent and showed maximal improvement of Th3-Th8 SCI cases after the second and consequent HSCs and HPs transplantations. After the first HSCs and HPs transplantation neurologic improvement was observed only in the cases of cervical injury, which can be explained by the fact that the first feeling of the slight changes in motor functionality (mostly of upper limbs) was much brighter in this category of the patients. By 5-8-th transplantations the quadriplegics were able to turn in their beds independently, the strength in upper extremities and back increased, and they did not require fixation to a wheelchair with the belts or any other devices. However, three years after the first transplantation, the most positive results were observed in lumbosacral cases and, strangely enough, in cervical SCI. At least, the improvement of life quality was more obvious in quadriplegics, both for the patient and for their relatives.

Accordingly, these data report more vigorous repair of motor functions at Th3-Th8 level of SCI after HSCs and HPs transplantations. Although, the represented data show limited opportunity for the restoration at the level of cervical and lumbar enlargement, we observed the benefits of cell transplantations at these levels. Follow-up of the SCI patients after the HSCs and HPs transplantations demonstrated neurologic progress in 61.1%, and it was associated with strengthening of the muscles, development and/or increase of motor activity, regress of sensitive disorders, and improvement of bowel and bladder functions. The most notable clinical effect was achieved in locomotion. In most cases, the changes in motor functions were minimal after the first HSCs and HPs transplantation and manifested in



	Baseline	After 1 transplatation	After 2 transplatation	After 20 transplatation
Complete	74.9	79.9	81	79.3
Incomplete	142.1	150.5	172.6	182.6

Figure 5 Motor function restoration after hematopoietic stem cells and hematopoietic precursors transplantation in complete spinal cord injury patients and incomplete. [a]*P* < 0.05 as *vs* the baseline; [c]*P* < 0.05 as *vs* the score after the first transplantation; [b]*P* < 0.1 as *vs* the baseline.

lightweight positions. Further intensive rehabilitation led to strengthening of extremities muscles, increase of pace and range of movements during exercise tests. After the second HSCs and HPs transplantation 33 patients were able to stabilize knee joints, to stand in knee supporting position independently and developed some elements of walking with assisting devices (walkers). It should be noted that 96% of the patients demonstrated no signs of neurologic restoration for several years before HSCs and HPs therapy. One of the patients from the United States restored independent automatic walk in a month of the therapy that included 4 administrations of the HSCs and HPs, and left the hospital on their own feet, although their previous treatment in the United States lasted 5 years. The similar recovery was observed in the patient from Bosnia and Herzegovina, when two administrations were enough to restore the walking function after 6 years of ineffective therapies in various clinics of the world.

Post HSCs and HPs transplantation changes of motor activity depending on the type of injury

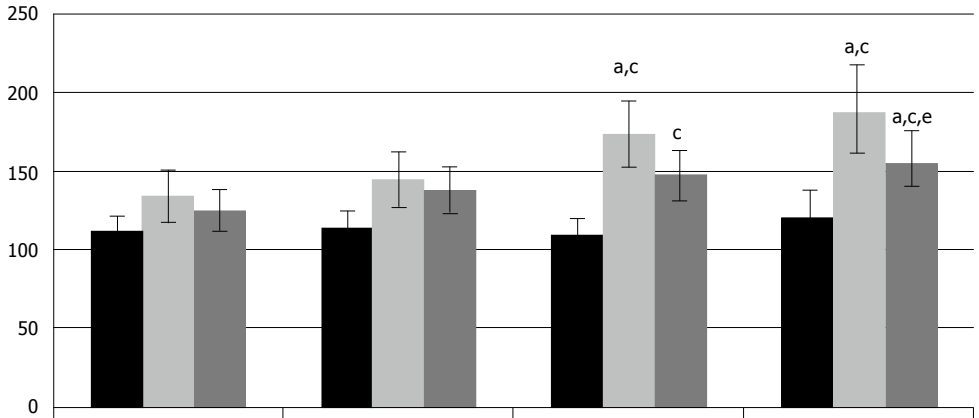
As expected, comparison of the results, depending on type of injury, showed better progress in the cases of incomplete SCI. Sixty percent of incomplete injury cases demonstrated improved locomotion, as compared to 46.7% of complete SCI cases (Figure 5). The patterns, identified at early period of the therapy, were fully confirmed 1-3 years post therapy beginning. They are supported by the changes of clinical condition in incomplete SCI cases, manifested in the increase of motor points from baseline 142.1 ± 5.7 to 150.5 ± 5.7 after the first transplantation, and 172.6 ± 8.1 after the second transplantation ($P < 0.05$) (Figure 5). In complete SCI cases neurologic improvements were minimal and made only 5 points after the first HSCs and HPs transplantation ($P < 0.05$). The tendency to improve to 81 ± 7.9 points was observed after the second transplantation ($P < 0.1$), which can be explained by the insignificant number of cases ($n = 11$); Due to different baseline scores of incomplete SCI and complete SCI cases, the comparison between the stages of therapy was done in percent and did not demonstrated significant difference in results after the first, or after the second, and even after the twentieth HSCs and HPs transplantations.

Post HSCS AND HPS transplantation changes of motor activity depending on years post injury

The increase of motor activity increase (Figure 6) after HSCs and HPs transplantation was observed only in the cases of 2-5 years post SCI; it was manifested in the motor activity increase from baseline 134.5 ± 7.3 points to 144.5 ± 8.6 points after the first transplantation and to 173.4 ± 10.7 after the second $P < 0.05$ between baseline and transplantations, respectively). Neither cases of 1-2 years post SCI, nor the cases over 5 years post injury showed statistically significant changes of clinical symptoms. These results seem to be conditioned by the inability of HSCs and HPs to realize their regeneration potential, due to residual inflammation and apoptosis in the patients with the period post SCI, varying from 1 to 2 years and due to degenerative changes in spinal cord in over 5 years old SCI cases. Still, regress of motor neurologic symptoms was observed in some of the patients with such SCI, so that in one of the cases the motor functions were considerably repaired 29 years post injury.

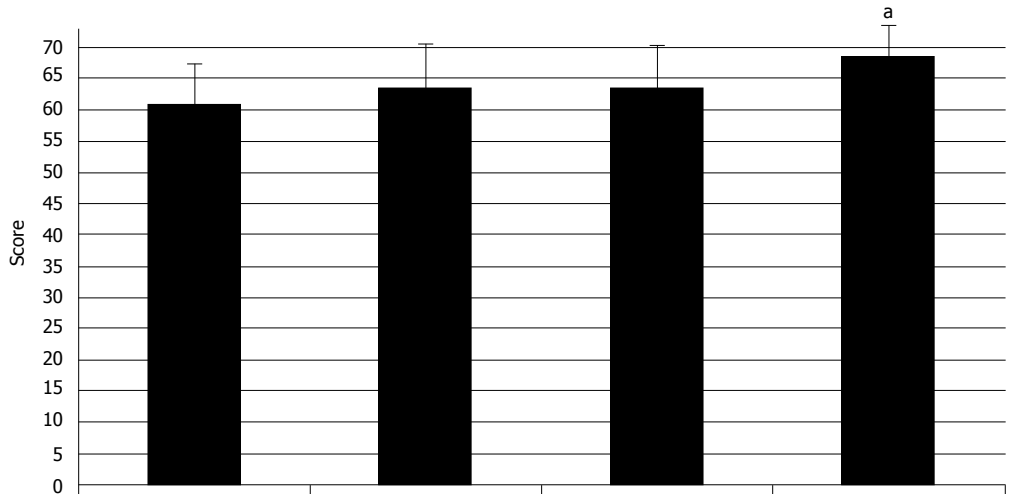
Testing muscle strength repair in SCI patients after HSCS AND HPS transplantation with Medical Research Council Scale

The Medical Research Council Scale was used to confirm the obtained results of motor progress after the HSCs



	Baseline	After 1 transplatation	After 2 transplatation	After 20 transplatation
From 1 to 2 yr	111.1	114	109.4	120.6
From 2 to 5 yr	134.2	114.5	173.4	186.7
From 5 yr and more	125.2	138.1	147.3	154.5

Figure 6 Clinical progress of motor restoration after hematopoietic stem cells and hematopoietic precursors transplantation depending on years post injury. $^{a}P < 0.05$ as *vs* the baseline; $^{c}P < 0.05$ as *vs* the score after the first transplantation; $^{e}P < 0.05$ as *vs* the group of patients with 2-5 years post injury.



	Baseline	After 1 transplatation	After 2 transplatation	After 20 transplatation
Points	60.9	63.6	63.5	68.6

Figure 7 Muscle strength restoration evaluated by Medical Research Council Scale after hematopoietic stem cells and hematopoietic precursors transplantation. $^{a}P < 0.05$ as *vs* the baseline.

and HPs transplantation in chronic SCI patients. The scale seems to be one of the most convenient and clear measurements of the strength of separate muscles, and originally was meant to detect locomotion deficit in the injuries of peripheral nerves. Total score for the absence of neurologic impairment makes 100 points.

As seen in Figure 7, the HSCs and HPs transplantation, accompanied by intensive rehabilitation, resulted in the increase of the muscle strength at all stages of research ($P < 0.05$). The second HSCs and HPs transplantation did not lead to muscle strength increase in damaged extremities. These data can be explained by insensitivity of the measurement tool to paresis improvements, the so called ceiling effect, that agrees with the data of Belova[11]. It is also confirmed by the analysis of muscle strength, the patients being distributed according to the level and type of injury (Figure 8). Strengthening of the muscles was observed in the cases of more severe injuries: at the level of cervical intumescence and with complete SCI.

On the other hand, recovery of the muscle strength after HSCs and HPs transplantation repeated the pattern of the progress of motor functions, depending on the years post injury. This manifested in the slight

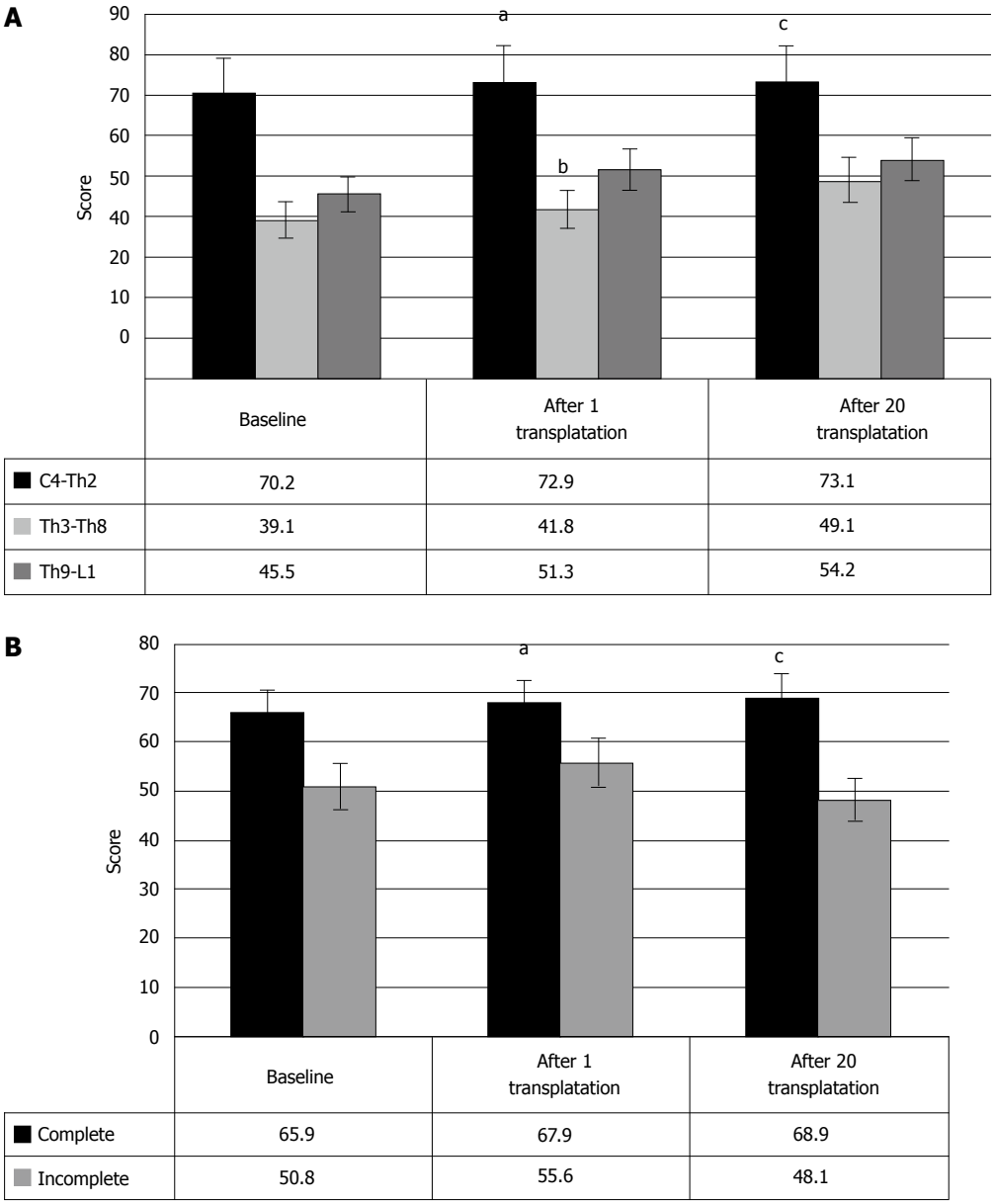


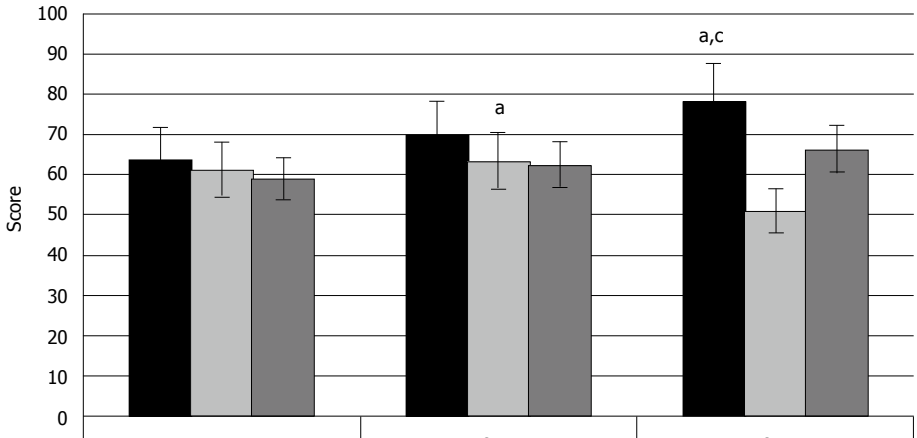
Figure 8 Clinical picture of muscle strength restoration evaluated by the Medical Research Council Scale after Hematopoietic stem cells and Hematopoietic precursors transplantation in the patients with different levels of spinal cord injury (A) and complete and incomplete spinal cord injury (B). [a]*P* < 0.05 as *vs* the baseline; [c]*P* < 0.05 as *vs* the score after 1 HSCs and HPs transplantation; [b]*P* < 0.1 as *vs* the baseline. HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors.

score increase in the cases of 2-5 years post SCI after the first HSCs and HPs transplantation (from baseline 63.8 ± 4.6 points to 78 ± 7.1 points after HSCs and HPs transplantation, $P < 0.05$, respectively). However, after the second HSCs and HPs transplantation, muscle strength increase was registered only in the patients with 1-2 years old injury. The cases of over 5 years old SCI demonstrated no statistically valid increase of muscle strength, herewith, confirming the hypothesis of hindered motor restoration, due to degenerative changes in spinal cord in these cases. Hence, the changes in muscle strength, measured by Medical Research Council Scale, demonstrated improvement of locomotion after HSCs and HPs transplantation despite low sensitivity of the tool and consequent low increase of the score (Figure 9).

Sensation repair in SCI patients after HSCs and HPs transplantation

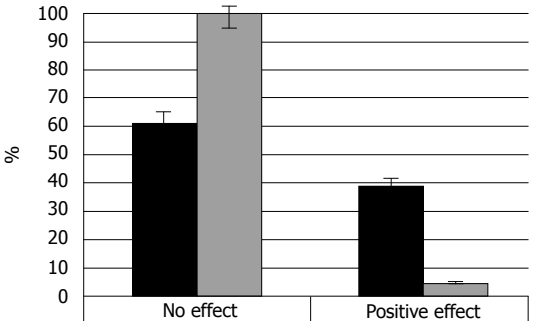
Sensation repair after the intrathecal transplantation of HSCs and HPs was evaluated in 71 patients by the assessment of neurologic condition that included 2-point tests of pain, temperature, deep sensation on dermatomes on both sides, as well as the assessment of the feeling of muscle "heaviness" in rest and after exercise in upper and lower extremities, abdomen and back. Total score, denoting absence of neurologic motor disorders, made 312 points.

As different from the locomotion, the repair of sensation was registered in a much fewer number of chronic



	Baseline	After 1 transplatation	After 20 transplatation
From 1 to 2 yr	63.8	69.8	78
From 2 to 5 yr	61.3	63.3	50.9
From 5 yr and more	58.9	62.3	66.3

Figure 9 Clinical picture of muscle force restoration evaluated by Medical Research Council Scale after hematopoietic stem cells and hematopoietic precursors transplantation depending on years post injury. $^{a}P < 0.05$ as *vs* the baseline; $^{c}P < 0.05$ as *vs* the score after 1 HSCs and HPs transplantation. HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors.



	No effect	Positive effect
Experimental arm	61.2	38.8
Control	100	4.8

Figure 10 General efficiency of sensation restoration after hematopoietic stem cells and hematopoietic precursors transplantation.

SCI cases (Figure 10), the reason as yet remaining unclear. At the same time, the analysis of the obtained clinical data showed (Figure 11A) that the cell therapy led to the increase of sensitivity from baseline 124.3 points to 138.4 after the first and to 153.5 points after the second transplantations of HSCs and HPs ($P < 0.05$, between the stages of research).

Clinically, the repair manifested in the expansion of sensation areas, accompanied by gradual involvement of new dermatomes. Major part of the patients observed the elements of deep sensation after the first transplantation and characterized them as the "heaviness" of muscles in rest and after physical training. Further, it was noted that development of the feeling of the position of lower extremities in space preceded stabilization of knee joints and development of the first elements of walking.

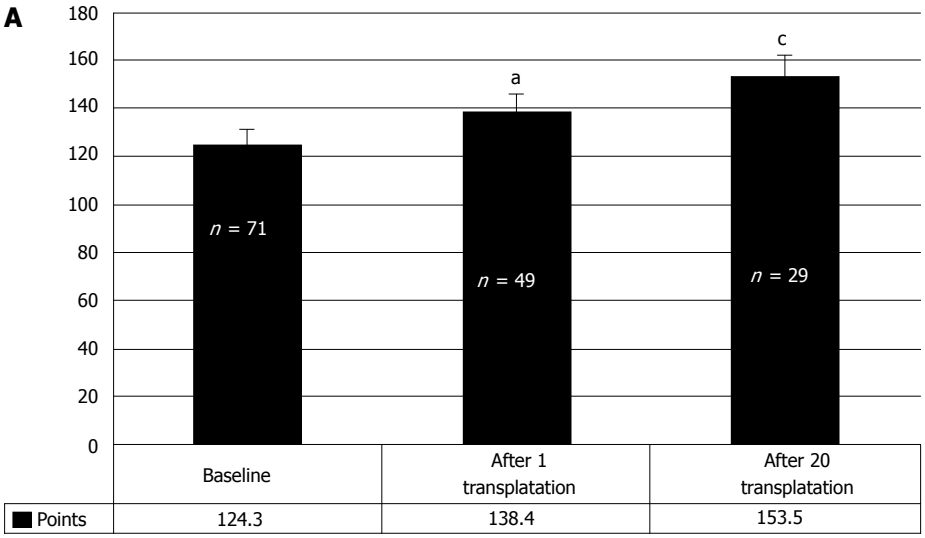
Expansion of the areas of surface sensation did not depend on the level of injury, *i.e.,* the sensation could manifest with separate dermatomes of lower and/or upper extremities, anterior chest or abdomen walls. In most of the cases the dermatomes did not restore in full, but only partially the sensation seldom restored unilaterally. Having received 5-7 HSCs and HPs transplantations, some of the patients restored sensation in all or almost all dermatomes of extremities and body. Hence, after the transplantation of HSCs and HPs, the sensation restores in chronic SCI cases, but in fewer cases than locomotion.

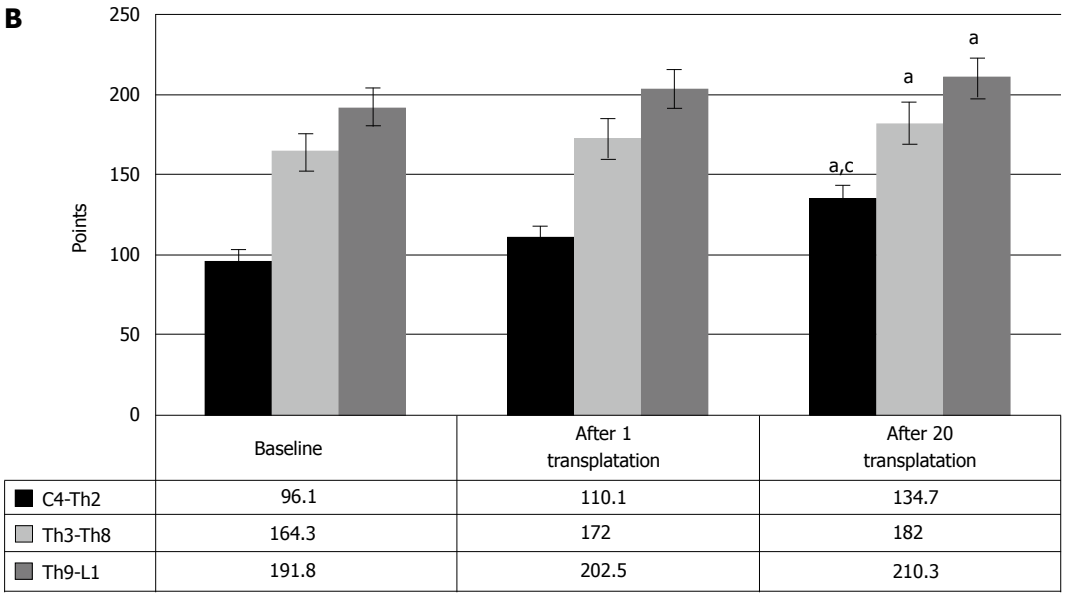
Case distribution, depending on the level or type of injury, demonstrated restoration of sensation in the most severe cases (complete SCI of cervical intumescence) (Figures 11B and C). These results are likely to be conditioned by low sensitivity of the measurement scale, *i.e.*, "ceiling effect". However, gradation of the sensation disorders was copied from widely applied measurement scales, including ISCSCI-92, and, hence, demonstrated the inefficiency of applied evaluation methods that demand upgrade.

No clinical changes were observed in the distribution of the cases, depending on the years post injury. This can be explained by lesser damage of posterolateral parts of spinal cord that agrees with the multiple data of pathomorphological tests. However, additional tests are necessary to confirm this hypothesis. Obtained clinical data of sensation repair were objectified with SSEP[12].

Evaluation of bladder repair in SCI patients after HSCs and HPs transplantation

Efficacy of the rapier of bladder functions was evaluated





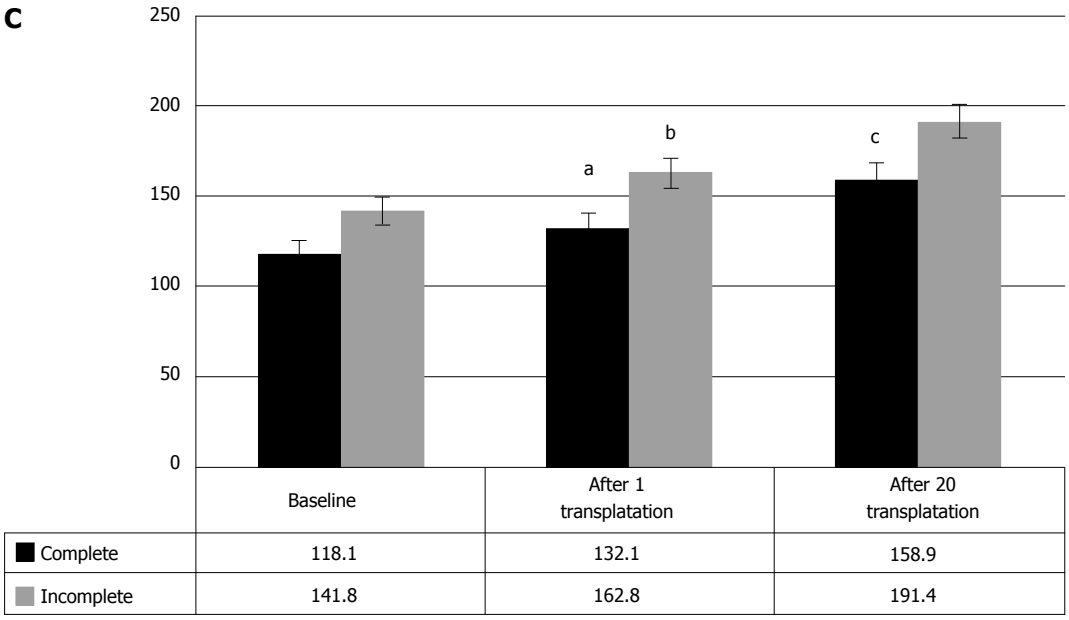


Figure 11 Clinical changes in sensation after hematopoietic stem cells and hematopoietic precursors transplantation (A) in the spinal cord injury patients with different levels of injury (B) and in the patients with complete and incomplete spinal cord injury (C). [a]*P* < 0.05 as *vs* the baseline; [c]*P* < 0.05 as *vs* the score after 1 transplantation; [b]*P* < 0.1 as *vs* the baseline.

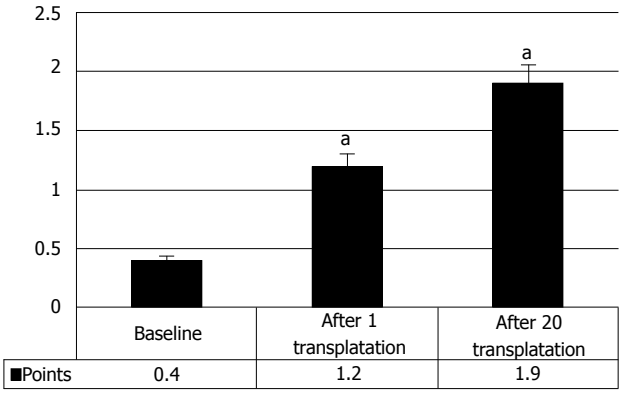


Figure 12 Clinical progress of urinary restoration after hematopoietic stem cells and hematopoietic precursors transplantation. $^{a}P < 0.05$ as *vs* the baseline.

in 72 patients with the assessment of neurologic condition that included 3-point assessment of the feeling of urination and 5-point assessment of urine retention. Total score that denotes absence of neurologic signs of urinary disorder is 8 points.

Repair of the urinary system was observed in 47.7% of the cases after the intrathecal transplantation of HSCs and HPs. Clinically, the restoration of urinary system manifested in creeping sensation in the body or unpleasant feelings in the lower abdomen that preceded involuntary urination, but complete syndrome of vegetative hyperreflexia was absent (changes in blood pressure and heart rate, arrhythmia, sweating, fever above the injury level). Many patients observed the feeling of weak "swelling" above pubic symphysis that allowed beginning of bladder training with closing urethral or cystostomic catheter. Further restoration of the capacity to retain urine for at least 1-3 min led to intermittent catheterization, or refusal from the cystostomy. In some cases, 3-5 intrathecal transplantations of HSCs and HPs resulted in full refusal from intermittent catheterizations and further complete repair of urinary function.

Analysis of the clinical data showed that in 33.8% cases the manifestations of urinary restoration began after the first transplantation of HSCs and HPs, showing clinical improvement from baseline 0.4 ± 0.2 points to 1.2 ± 0.2 points after the first transplantation of HSCs and HPs ($P < 0.05$) (Figure 12). Consequent transplantations improved the urinary function further, thus increasing the score to 1.9 ± 0.4 points.

Hence, the transplantation of HSCs and HPs can lead to gradual restoration of urinary function in chronic SCI cases. Analysis of the data, depending on the level of injury (Figure 13), showed that largely, the improvement in the urinary system after HSCs and HPs transplantation was noted at Th3-Th8 level of SCI and at the level of lumbar enlargement (70%). It manifested in the increasing urinary restoration (Figure 13) from baseline 1.1 ± 0.8 points to 2.5 ± 0.8 points after the first transplantation and to 2.9 ± 0.9 points after the repeated HSCs and HPs transplantations ($P < 0.05$ between the therapy stages) in Th3-Th8 SCI cases. In SCI at the level of lumbar enlargement the urinary function changed from baseline 1 point to 1.9 and 2.8 after the first and the second transplantations, respectively ($P < 0.05$ between the therapy stages).

Despite fewer number of the SCI patients at the level of cervical intumescence, who showed the urinary system repair (36.8%), the restoration from baseline 0.1 points to 0.7 points and to 1.3 points was clinically registered after the first HSCs and HPs transplantation after the second HSCs and HPs transplantation, respectively ($P < 0.05$ between the therapy stages).

Thus, the urinary system after the intrathecal transplantation of the HSCs and HPs restores irrespective of the level of the SCI. However, the urinary system restores more efficiently in the cases of SCI at the level of Th3-Th8 and lumbar enlargement.

The repair of the urinary system after HSCs and HPs transplantation did not depend on the type of SCI, as shown in Figure 14A. However, in the cases of the incomplete SCI the urinary disorder at a baseline was less significant, as well as after the first transplantation. After the second transplantation, no statistically significant changes in the clinical evaluation of urinary system have been observed.

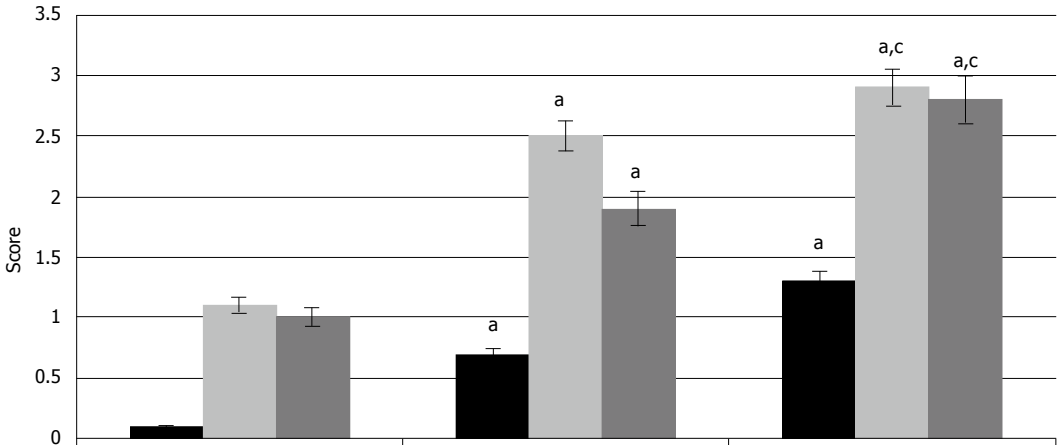
Restoration of the urinary system did not depend on period post injury, either. As seen in Figure 14B, some restoration of urinary function was observed irrespectively from years post injury. There is a clear tendency for further improvement of urinary function after 2 or 3 years of HSCs and HPs therapy, as compared to baseline.

Consequently, the intrathecal transplantation of HSCs and HPs in chronic SCI patients is an efficient method to repair urinary function. The lower levels of SCI are more prone to restore urinary function, which can be explained by closer location of urination centers in sacral spinal segments to lesion sites and, possibly, by larger concentration of HSCs and HPSs in the sites of injury. Herewith, neither the type of injury, nor years post injury, do not influence restoration of urinary function.

Evaluation HSCS and HPS transplantation efficiency in SCI patients with ASIA, FIM and ISCISCI-92

The functional repair of spinal cord was analyzed for 72 chronic SCI cases; it was measured with ASIA, ISCISCI-92 and FIM indexes at all stages of HSCs and HPSs transplantation. The evaluation with ASIA index demonstrated regress of neurologic symptoms only in 23 cases. Two patients with complete SCI (ASIA A) showed restoration of the locomotion below neurologic level of injury with muscle force of no less than 3 points (ASIA C) after HSCs and HPs transplantation, and over 3 points (1 patient, ASIA D). The ASIA B patient after HSCs and HPs transplantation showed neurologic restoration to ASIA C.

Nevertheless, the above shown analysis of clinical



	Baseline	After 1 transplatation	After 20 transplatation
C4-Th2	0.1	0.7	1.3
Th3-Th8	1.1	2.5	2.9
Th9-L1	1	1.9	2.8

Figure 13 Clinical changes of urinary function after hematopoietic stem cells and hematopoietic precursors transplantation at different levels of spinal cord injury. $^{a}P < 0.05$ as *vs* compared to the baseline; $^{c}P < 0.05$ as *vs* the score after the first transplantation.

regress of neurologic symptoms demonstrates inefficiency of the ASIA impairment scale that was used to evaluate restoration of the spinal cord functions. On the one hand, it is associated with the specific features of restoration of spinal cord functions, and on the other, with low sensitivity of the index that gives only general estimation of regress of the neurologic damage. According to Belova[11] the ASIA index is applicable only for screening of the spinal functions during acute period of SCI. To evaluate neurologic progress in SCI, the more detailed characterization of locomotion, sensation and urination is required in every individual case.

As shown above, the sensation recovered after the manifestations of the restoration of motor functions analysis, especially in S4-S5 segments. The sensation restored mosaically, frequently after the development of passive or active movements, and involved the segments only partially. Absence of sensation in S4-S5 segments conditioned ASIA A level of impairment, even if motor functions of certain muscles below injury level were preserved to a certain extent. In this respect, 10 patients observed restoration of muscle force in most of the muscles below the level of injury that enabled their walking with assisting devices after 4-8 transplantations, while currently, two patients are able to cover short distances independently. However, only one of these patients demonstrated restoration of sensation in S4-S5 segments.

Hence, the ASIA impairment scale is effective to assess the degree of disability, but is ineffective, when used to assess the restoration of spinal cord functions in chronic SCI after HSCs and HPs transplantation.

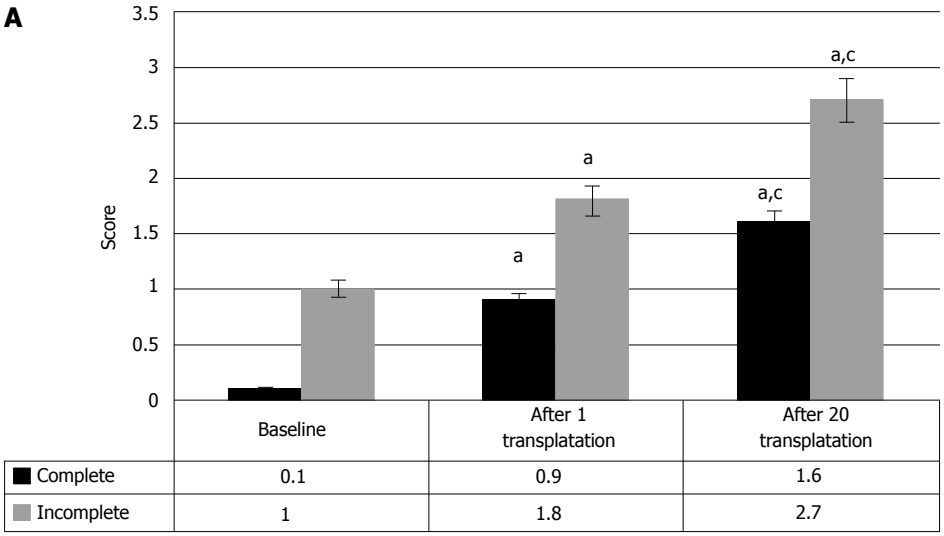
Motor progress was also assessed with the ISCISCI-92 motor and sensory scores, and the data coincided with those received in evaluation of motor functions by the specifically developed scale. The number of the patients with the signs of locomotive repair was 56.9%. Moreover, the motor activity rates increased from baseline 32.7 points to 37.1 after the first transplantation and to 39.9 after repeated transplantation of HSCs and HPs ($P < 0.05$, at each stage of transplantation) (Figure 14C).

In spite of clinical restoration of sensation in 38.6% of the patients, the ISCISCI-92 scores did not confirm these data. This is conditioned by the absence of evaluation of deep sensation in ISCISCI-92, and, as noted before, by the "ceiling effect", when the neurologic status changes within the partial restoration of sensation.

Hence, the assessment of motor restoration with the ISCISCI-92 scores demonstrated effectiveness of the HSCs and HPs transplantation in chronic SCI patients. The ISCISCI-92 score confirms the data of our specifically developed scale to assess the clinical motor restoration of spinal cord, thus, demonstrating its applicability in practice. The advantage of our evaluation scale of clinical motor restoration over the ISCISCI-92 lies in the multi-factor analysis of the motor activity, based on standard neurologic examination. Absence of changes in sensation as measured by ISCISCI-92 scores that, however, are accompanied by the clinical signs of restoration, demands development of new tools to measure changes both in surface sensation (touch and pain) and deep sensation. Despite partial solution of this issue in the specifically developed scale of clinical motor restoration, the "ceiling effect" was not overcome in partial restoration of this function.

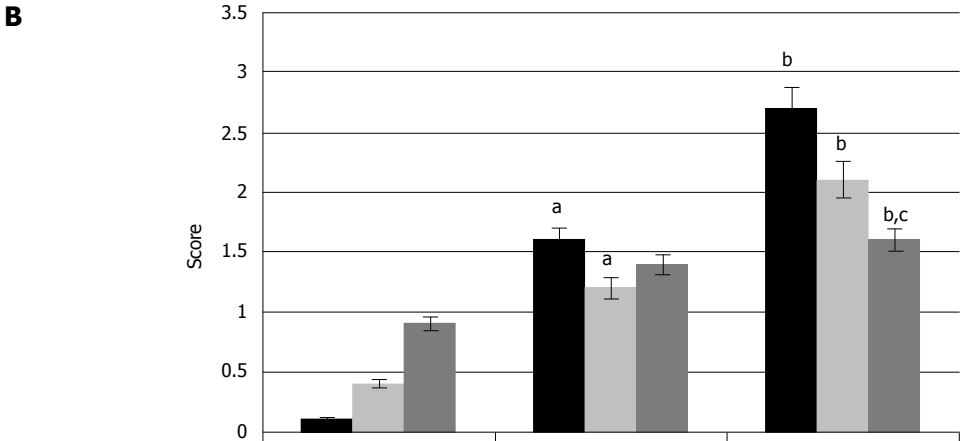
We would like to focus on the restoration of the

A



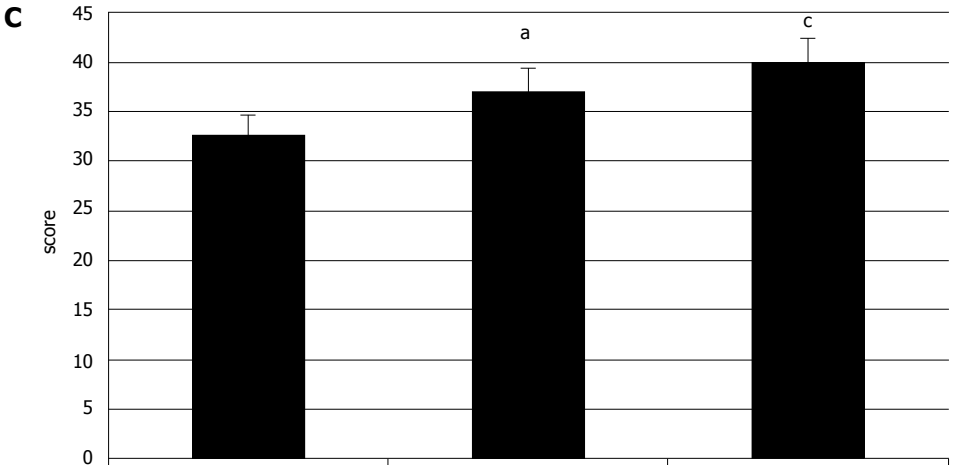
	Baseline	After 1 transplatation	After 20 transplatation
Complete	0.1	0.9	1.6
Incomplete	1	1.8	2.7

B



	Baseline	After 1 transplatation	After 20 transplatation
From 1 to 2 yr	0.1	1.6	2.7
From 2 to 5 yr	0.4	1.2	2.1
From 5 yr and more	0.9	1.4	1.6

C



	Baseline	After 1 transplatation	After 20 transplatation
Score	32.7	37.1	39.9

Figure 14 Urinary restoration after hematopoietic stem cells and hematopoietic precursors transplantation in complete/incomplete spinal cord injury patients (A) and depending on years post spinal cord injury (B); motor restoration after hematopoietic stem cells and hematopoietic precursors transplantation evaluated by ISCISCI-92 (C). [a]*P* < 0.05 as *vs* the baseline; [c]*P* < 0.05 as *vs* the score after 1 transplantation; [b]*P* < 0.1 as *vs* the baseline.

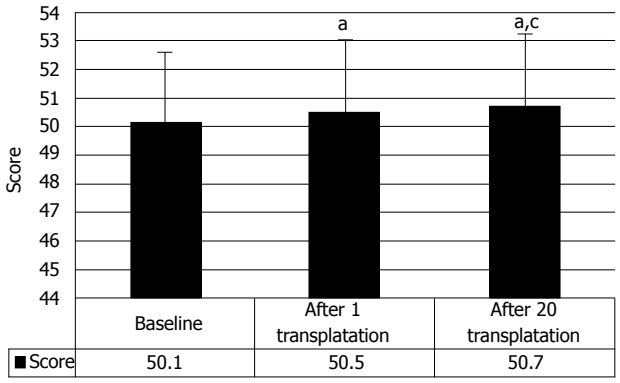


Figure 15 Changes of vital activity in the patients after hematopoietic stem cells and hematopoietic precursors transplantation measured by FIM. $^{a}P < 0.05$ as *vs* the baseline; $^{c}P < 0.05$ as *vs* the score after 1 HSCs and HPs transplantation. HSCs: Hematopoietic stem cells; HPs: Hematopoietic precursors.

functional independence after HSCs and HPs transplantation that was evaluated in 64 patients with the functional independence measurement (FIM) scale. The signs of the restoration of life activity was observed in 36.2% patients and were minimal (from baseline 50.1 points to 50.5 points after the first transplantation, and to 50.7 points after repeated HSCs and HPs transplantation; $P < 0.05$ at all stages of therapy, respectively) (Figure 15). It is associated with moderate restoration of the spinal functions after the first HSCs and HPs transplantations that manifested mostly in locomotion. However, as shown above, further transplantations resulted in more profound clinical progress. Besides, the FIM scale, when applied to chronic SCI cases has significant disadvantage: in the cases of considerable disorders of nerve impulse conductance, the FIM displayed very low sensitivity, due to absence of detailed functional evaluation. Accordingly, the analysis of the obtained data showed very slight improvement of the FIM scores, demonstrating improvement of the functional independence conditioned by the motor function of spinal cord.

Complications of intrathecal application of HSCS AND HPS

During 12 years of follow up we observed no life threatening complications resulting from the HSCs and HPs transplantation. The complications of HSCs and HPs administration were evaluated at three stages: stage 1 after the first transplantation; stage 2 after one year of the therapy that included 5.3 ± 0.5 administrations; stage 3 two years of regular administrations (10.1 ± 1.1). The complications were summed up in Table 4. We observed one case of cancer (femoral carcinoma) of 202 followed up cases. However, according to the conclusion of the experts of the Russian Cancer Research Centre, it was not associated with HSCs and HPs transplantation. Surprisingly, in the control group of 20 cases we registered one case of spontaneous brain cancer development (pituitary adenoma) too, for which the patient was operated on.

DISCUSSION

The therapy of SCI with autologous HSCs and HPs demonstrated high efficiency (to 95.1%) of stem ($CD34^{+}$) peripheral cells mobilization in SCI patients. It is well known that under the conditions of undamaged hematopoiesis, the hematopoietic stem cells (HSCs) circulate in peripheral blood of a human. But their concentration is extremely low (less than 0.01%) that makes their detailed study and transplantation almost impossible. High concentration of HSCs results from the damage of hematopoiesis (usually as a result of chemotherapy), or administration of colony-stimulating factors (CSF). In the clinical practice, the granulocyte CSF (G-CSF) and granulocyte macrophage CSF (GM-CSF) are the most widely used. These factors increase the concentration of HSCs 100-1000 times, thus allowing the harvest of the cells and their use for transplantation. It should be noted that mobilization of the stem cells and precursor cells into peripheral blood vessels in the patients with traumatic disease of spinal cord was efficient in all cases - both absolute number and the percentage of $CD34^{+}$ in leukoconcentrate received after 1 session of leukapheresis meet the transplantation standard of the number of mononuclears ($> 2 \times 10^{6}$/kg).

As seen from the description, we applied the suspe nsion of autologous HSCs and HPs, and not a standard suspension of autologous HSCs ($CD34^{+}$). We consider this cell suspension to reflect systemic specific response of bone marrow of each patient to the injury of the central nervous system, and the cell composition received in specific stimulation conditions and cryopreservation is unique and obligate. In case of stimulation of an SCI patient with G-CSF in the dose of 10 or 20 mg/kg for 5-6 d as it is recommended in the manuals of hematooncology, we harvest mature differentiated hematopoietic cells, able to restore hematopoiesis, and not injured nervous system. We applied the standard sparing scheme of simulation, which is ubiquitously used in pediatric oncology. This empirically selected mode of stimulation allowed us for new property of cell suspension that conditions its clinical effectiveness.

As seen from Table 3, we refused from cryopreservation with 10% DMSO with polyglycine, although the combination is considered ideal to protect HSCs. We applied lower concentration for cryopreservation, and, namely 5% DMSO and polyglycine that demonstrated its high efficiency and safety for intrathecal transplantation.

The stem cells and committed precursor cells form a so-called pool of HSCs. The expression of CD34 molecule on the surface of a membrane is common to all cells of the pool, and this property enabled use of flow cytometry methods to detect the precursor cells and to provide their quick count in any hematopoietic material. Last decades the peripheral blood was the main source of stem and precursor cells. Thus, for example, transplantation of separated fraction of mononuclear

Table 4 Clinical symptoms of the complications and side effects in spinal cord injury patients

Symptoms	Stages of research			Control group patients
	1 stage	2 stage	3 stage	
Increased spasticity	46%	49.9%	54.5%	0
Fever	15%	19%	18.8%	0
Post-puncture headache	11%	14.9%	12.2%	14.9%
High blood pressure	10%	8.1%	14.5%	0
Coordination disturbance	2.3%	1.3%	0	0
Dizziness	2%	3.4%	0	4.2%
Sleepiness	2.1%	1.7%	0	0
Emotional lability	1.6%	1.7%	0	0
Disordered consciousness	1.2%	0.8%	0.8%	0
Meningism	3.7%	2.95%	0.8%	0
Low blood pressure	1.68%	2.95%	5.9%	0
General % of the patients with complications	63.5%	72.9%	75%	19.1%

cells of peripheral blood with hematopoiesis stimulation permits considerable reduction of critical cytopenia in patients after high-dose chemotherapy. The phenomenon is conditioned by stem and precursor cells entering peripheral blood under colony stimulating factors influence. Special attention should be given to the composition of subpopulation of $CD34^+$ cells, that is, the number of the cells of different compartments of HSCs and HPs pool.

Subpopulation composition of $CD34^+$ cells was assessed by flow cytometry with triple-labeling method. Our analysis of efficiency of the suspension in SCI patients demonstrated that best motor restoration in SCI cases was observed only when the membrane of an autologous stem cell expressed gp130 protein. Gp130 is a transducing molecule of IL-6 cytokines and a receptor of cell functional condition. Basic pleiotropic action of these receptors is to contribute to cell differentiation, gene expression, stimulation or inhibition of cell growth and control of cell apoptosis. At day 4.5 and 5 of stimulation with G-CSF the abrupt decrease of gp130 expression was observed, which reduced activity of the cell preparation and therapy effect. The HSCs and HPs harvested according to standard protocol at day 6 of stimulation did not lead to any clinical effect. We received the pool of formed mononuclears with highly differentiated and well-diagnosed genuine hematopoietic and mesenchymal stem cells, therapeutic effect of which is disputable in our case. The cell suspension we use for therapy does not contain conventional HSCs, although they are assessed in $CD34^+$ gate, when evaluated in flow cytometer, the suspension contains heterogeneous mixture of mobilized low-differentiated precursors, promoting regeneration of nervous system. In this technique the dose has no relevance and can considerably vary. The standard cell composition is of the key importance, reflecting the level and concentration of the output of non-differentiated PC at the proposed sparing G-CSF stimulation modes in the patients with post-traumatic neurologic deficit. The proposed individual preparation contains the mixture of highly efficient mobilized stem precursor cells of bone marrow, including hematopoietic-like cells. To date, we are unable to accurately identify what exactly type of cells of this pool make the treatment effective, but this seems unimportant for the patients and the clinical practice. We know that using the proposed method of harvest, we receive a standard cell preparation that gives a steady, reproducible and progressing clinical effect.

This preparation has no prototype, as well as the presence or absence of HSCs ($CD34^+$) is not pivotal. Novelty of this preparation is determined by the presence of the mixture of non-differentiated cell precursors, restoring neurogenesis and regeneration in the damaged brain/spinal cord. The researches in cell medicine mention the facts of using HSCs to treat multiple sclerosis and amyotrophic lateral sclerosis. None of the authors used cryopreservation, they applied a single bolus injection of stem cell preparation. Our experience clearly demonstrated that only multiple and long-term (for 5-8 years) administration of the preparation will provide the maximal benefit of the existing regenerative potential of the cells and the opportunity to restore the damaged brain/spinal cord functions. It is the sparing 4 d long mode of G-CSF administration in the patients with neurologic deficit that provides for the harvest of all necessary nuclear cell precursors.

According to our evaluation of long-term outcomes of SCI cell therapy, the transplantation of autologous HSCs and HPs is an efficient method to repair lost functions in SCI patients, and it is not directly dependant on the dose and number of autologous HSCs and HPs transplantation. The patients with the lesion exceeding 50% of spinal cord cross-wise and 1 segment long-wise, and possibly those with moderate CSF circulation disorders should be excluded from therapy. Presumably, such patients require reconstructive surgical intervention with meningoradiculomyelolysis, spine stabilization and, possibly, tissue engineering of spinal cord. The HSCs and HPs transplantation only will hardly result in the restoration of spinal functions in these cases.

The rehabilitation is a requisite component in the therapy of chronic SCI patients.

Research of the SCI therapy demonstrated that to restore the functions, the conductance along various nervous pathways (pyramidal, extrapyramidal, spinothalamic, *etc*.) must be restored, and new synaptic links between injured segments of spinal cord must be established. Under these circumstances, the grey matter of spinal cord need not be replaced due to availability of cross innervation of dermatomes and myotomes in humans. Mere surgery and/or rehabilitation do not lead to the expected outcomes, as they do not eliminate the main cause of the disorder and do not restore injured neural structures of spinal cord. Application of the systems of adult stem and PCs confirmed the opportunity to restore spinal cord. The regulatory action of the mobilized progenitors, and not their regenerative potential, seem to be the main mechanism of functional restoration in SCI, activating synaptogenesis in adult brain, increasing plasticity of injured neural tissue of SC and developing functional neurophysiologic bypass. The intensity of HSCs and HPs regulatory potential depends on the size of SCI and directly proportional to intact neural structures of spinal cord.

The analysis of treatment efficiency depending on the level of injury deserves special attention. The reason for better clinical restoration at thoracic level seems to lie in morphological feature of the spinal cord structure and cervical and lumbar intumescence, where great number of neurons is located (second motor neurons, interneurons, *etc.*). The SCI at the level of intumescences leads to larger damage of spinal cell components and more intense pathologic processes; hence, the restoration in such cases of SCI is more difficult. The axons of motor neurons are located mostly at the thoracic level, the bodies of them are found in motor cortex of brain, and hence, less number of bodies of neural cells is involved into the injury. Restoration of the motor functions is associated with the increase of regeneration potential of the spinal cord, mainly at the level of cortical influence of HSCs and HPs on the intact bodies of motor neurons. The mechanism of HSCs and HPs effect does not seem to be associated with their differentiation into neurons and glial cells of SC. Most likely, the regulatory influence of HSCs and HPs at the site of SC injury leads to gene expression and secretion of neurotrophic factors, entailing growth and regeneration of axons in the site of injury and restoration of nerve impulse conductance along the intact but functionally inactive axons. As a result, the available ensembles of neurons are differentiated due to the development of new synaptic contacts below and above the injury site. The phenomenon is only observed when the stem cells are transplanted into the injured spinal cord, and it fully agrees with the data offered by Snyder[13]. The development of new synaptic links below and above injury level can serve as an explanation of the clinical results of motor restoration that we have observed.

The analysis of the obtained data indirectly confirms the hypothesis of HSCs and HPs influence on axonal growth in the site of injury or development of conductance along functionally inactive, but anatomically intact fibers, as it is the patients with incomplete injury, who demonstrate maximal restoration of motor functions. Consequently, incomplete SCI is prognostically more favorable for restoration of motor functions of spinal cord. However, to obtain representative results the clinical data have to be compared depending on the level of SCI. In the cases of complete SCI we observed intensively restoring functions, too.

The issue of termination of HSCs and HPs therapy of SCI remains important for us. Many patients, who have completed 3 year and 6 year courses, insist on continuation of the therapy. Their arguments are quite simple: "My own cells cannot hurt me and I see steadily increasing positive effect from them, so it is harmless to continue the therapy". To date, 15 patients received HSCs and HPs transplantation for 8 years on a regular basis and no negative effects have been observed either at the level of clinical picture, or at the level of thorough paraclinical examination.

Summing up, we can conclude that the method is safe, effective and considerably improves the life quality of SCI patients. Administration of the autologous cell systems of hematopoiesis precursors led to real restoration of various movements and improved life quality in major part of our patients. About 15 patients are able to walk independently or with supporting devices, over half of them restored sensation of different types and the function of the bowel and bladder. The therapy was approved for clinical use as the treatment of choice. In terms of the long-term clinical outcomes, we can discuss complexity of the processes, observed in the central nervous system after SCI and under HSCs and HPs therapy, which are often hard to explain from clinical point of view. Being limited by the size of journal article, we are unable to demonstrate the whole range of long-term neurophysiologic and urodynamic paraclinical results, and their correlations with the mentioned clinical data, but we would be happy to offer them in our other works.

COMMENTS

Background

Contemporary healthcare have greatly improved the survival rates in spinal cord injuries (SCI) cases as well as their life expectancy, leading to the overall growth of the national economic burden. However, current healthcare advances have not led to any breakthrough in restoration of the functions of spinal cord after the injury, and ever since the Edwin Smith Papyrus the SCI has been classified as the ailment not to be treated. To date SCI is a verdict that entails impossibility to return to previous way of life, to restore previous working capacity and reproductive functions, resulting in tremendous social and economic losses. Inefficiency of the available SCI therapies used to be explained by the absence of the regeneration potential in adults, and the restoration of the damaged neural cells has been demonstrated only recently.

Research frontiers

By now, the first steps to develop new restorative therapy of SCI have been made, and the cell transplantation is the most obvious choice, although no universally acknowledged methods to restore spinal cord after the injury are

observed. The methods of transplantation and the types of cells significantly vary; the evidence gathered is mostly limited by a one or two years follow up. Being involved into stem cell transplantation for SCI for about 25 years in research and in clinical practice we have accumulated substantial experience of achievements and failures in stem cell therapy. In the current work, the authors describe the cell therapy that proved the safest and the most effective both in the short-term period and in long-term follow-up.

Innovations and breakthroughs

The method implies multiple long-term transplantations of the preparation of hematopoietic stem cells and hematopoietic precursors that was harvested from peripheral blood after sparing mode of administration of granulocyte colony-stimulating factor. The composition of the applied preparation is characterized. The cells are administered intrathecally in a subarachnoid space every three months and the transplantation is followed by vigorous specialized rehabilitation. The effects are evaluated by conventional indexes and tests, including somatosensory evoked potentials tests and urodynamic tests, as well as by specifically developed scales. The effects of 20 consecutive transplantations for each case are measured.

Applications

The method is safe, effective and is applicable to chronic SCI cases when no further restoration of the functions is observed. It considerably improves the life quality of the SCI patients. The method received official approval in the Russian Federation in 2005 and in 2006 and is recommended as the therapy of choice.

Terminology

Intrathecal transplantation means the infusion of the cells in the subarachnoid space in the course of lumbar puncture.

Peer-review

This is an important manuscript describing the clinical outcome of cellular therapy for spinal cord injury.

REFERENCES

1 **Lee BB**, Cripps RA, Fitzharris M, Wing PC. The global map for traumatic spinal cord injury epidemiology: update 2011, global incidence rate. *Spinal Cord* 2014; **52**: 110-116 [PMID: 23439068 DOI: 10.1038/sc.2012.158]

2 **National Spinal Cord Injury Statistical Center**. Spinal cord injury facts and figures at a glance. *J Spinal Cord Med* 2014; **37**: 243-244 [PMID: 24559421 DOI: 10.1179/1079026814Z.000000000260]

3 **Noonan VK**, Fingas M, Farry A, Baxter D, Singh A, Fehlings MG, Dvorak MF. Incidence and prevalence of spinal cord injury in Canada: a national perspective. *Neuroepidemiology* 2012; **38**: 219-226 [PMID: 22555590 DOI: 10.1159/000336014]

4 **DeVivo MJ**. Causes and costs of spinal cord injury in the United States. *Spinal Cord* 1997; **35**: 809-813 [PMID: 9429259]

5 **Raisman G**. Sniffing out new approaches to spinal cord repair. *Nat Med* 2000; **6**: 382-383 [PMID: 10742141 DOI: 10.1038/74638]

6 **Huang H,** Chen L, Sanberg P. Cell Therapy From Bench to Bedside Translation in CNS Neurorestoratology Era. *Cell Med* 2010; **1**: 15-46 [PMID: 21359168]

7 **Lima C**, Escada P, Pratas-Vital J, Branco C, Arcangeli CA, Lazzeri G, Maia CA, Capucho C, Hasse-Ferreira A, Peduzzi JD. Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. *Neurorehabil Neural Repair* 2010; **24**: 10-22 [PMID: 19794133 DOI: 10.1177/1545968309347685]

8 **Zorin VL**, Cherkasov VR, Zorina AI, Deev RV. The Characteristics of World Market Cell Technologies. *Kletochnaya Transplantologiya i tkanevaya engineeriya* 2010; 3: 96-115 (in Russian)

9 **Bryukhovetskiy AS**. Transplantatsiya nervnikh kletok i tkanevaya engineeriya mozga pri nervnikh bolezniakh [Transplantation of nerve cells and tissue engineering of brain in nerve diseases]. Moscow, ZAO Neurovita, 2003: 1-400 (in Russian)

10 **Bryukhovetskiy AS**. Travma spinnogo mozga: kletochniye tekhnologii v lechenii i reabilitatsii [Spinal cord injury: Cellular technologies in the treatment and rehabilitation]. Moscow, Prakticheskaya meditsina, 2010: 1-341 (in Russian)

11 **Belova AN**. Neiroreabilitatsiya: Rukovodstvo dlya vrachei [Neurorehabilitation: A Manual for Physicians]. Moscow: Antiodor, 2000: 1-736 (in Russian)

12 **Frolov AA**, Bryukhovetskiy AS. Effects of hematopoietic autologous stem cell transplantation to the chronically injured human spinal cord evaluated by motor and somatosensory evoked potentials methods. *Cell Transplant* 2012; **21** Suppl 1: S49-S55 [PMID: 22507680 DOI: 10.3727/096368912X633761]

13 **Snyder E**. Neural stem cells: Developmental insights may suggest therapeutic options. Proceedings of the 7th International Congress of the Cell Transplant Society; 2004, November 17-20; Boston, MA, USA, 2004: 53

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ORIGINAL ARTICLE

Retrospective Study

Cytomegalovirus reactivation after autologous stem cell transplantation in myeloma and lymphoma patients: A single-center study

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Institutional review board statement: The study was approved by the institutional Ethical Committee without a formal document, considering that all patients had signed an informed consent granting use of sensitive data for scientific purposes at time of admission in our Institute.

Informed consent statement: All the patients had signed an informed consent granting use of sensitive data for scientific purposes at time of admission in our Institute.

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Abstract

AIM: To determine the incidence of and the risk factors for cytomegalovirus (CMV) symptomatic infection and end-organ disease after autologous stem cell transplantation (ASCT).

METHODS: A total of 327 consecutive non CD34$^+$ selected autografts performed from the Hematology and Stem Cell Transplantation Unit of Regina Elena National Cancer Institute of Rome (Italy) in the period comprised between January 2003 to January 2015, were reviewed. Over the 327 autografts, 201 were performed in patients with multiple myeloma, whereas the remaining 126 in patients affected by non-Hodgkin's lymphoma and Hodgkin's lymphoma. The patients who underwent an ASCT for an acute leukemia (n = 20) in the same

period were excluded from this analysis. CMV DNA load in the blood has been determined by polymerase-chain reaction in the case of a clinical suspicion of reactivation, therefore, no routine monitoring strategy was adopted. In the presence of signs and symptoms of CMV reactivation an antiviral treatment was performed.

RESULTS: Overall, 36 patients (11%) required a specific antiviral treatment for a symptomatic CMV reactivation (n = 32) or an end-organ disease (n = 4). We observed 20 and 16 cases of CMV reactivation among lymphoma (16%) and myeloma patients (8%), respectively. Among cases of end-organ disease, 3 were diagnosed as interstitial pneumonia and one remaining case as hemorrhagic enteritis. All cases of CMV reactivation were observed in IgG seropositive patients, with no documented cases of primary CMV infection. All patients were treated with a specific antiviral therapy, with a global rate of hospitalization of 55%; four patients received intravenous immunoglobulins. Transplant-related mortality was significantly higher in patients who experienced a CMV reactivation (8.4% ± 4.7% *vs* 1.7% ± 0.8%; P = 0.047). In univariate analysis, a pre-transplant HBcIgG seropositivity, a diagnosis of T-cell non-Hodgkin's lymphoma and higher median age at transplant were significantly associated with the risk of developing a clinically relevant CMV infection requiring specific antiviral therapy (P < 0.001, P = 0.042 and P = 0.004, respectively). In multivariate analysis, only a pre-transplant HBcIgG seropositivity (OR = 8.928, 95%CI: 1.991-33.321; P = 0.023) and a diagnosis of T-cell non-Hodgkin's lymphoma (OR = 4.739, 95%CI: 1.511-11.112; P = 0.042) proved to be independent predictors of a post-transplant clinically relevant CMV reactivation.

CONCLUSION: A symptomatic CMV infection can occur in about 11% of adult patients with lymphoma or myeloma undergoing ASCT. A pre-transplant HBcIgG seropositivity and a diagnosis of T-cell non-Hodgkin's lymphoma should be considered as independent predictor factors of CMV reactivation.

Key words: Cytomegalovirus; Autologous hematopoietic stem cell transplantation; Lymphoma; Myeloma; HBcIgG seropositivity; Transplant-related mortality

Core tip: Data about cytomegalovirus (CMV) reactivation in autologous hematopoietic stem cell transplantation (ASCT) are limited. We performed a retrospective observational study on 327 autografts consecutively performed for lymphoma (n = 126) or myeloma (n = 201) patients in our Institution. Aim of the study was to determine the incidence of and the risk factors for CMV symptomatic infection and/or end-organ disease, defined according to published recommendations, and the impact on Transplant-Related Mortality. Our data show that a symptomatic CMV infection can occur in about 11% of adult patients with lymphoma or myeloma undergoing ASCT. Most of cases of CMV reactivation are easily manageable but it can be a potentially life-threatening complication. As for risk factors, a pre-transplant HBcIgG seropositivity and a diagnosis of T-cell non-Hodgkin's lymphoma should be considered as independent risk factors for CMV reactivation after ASCT.

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INTRODUCTION

Cytomegalovirus (CMV) reactivation is not uncommon and could determine a CMV-related disease in immuno-compromised patients. CMV disease may involve almost any organ, particularly lung and gastrointestinal tract. CMV reactivation and end-organ disease after allogeneic hematopoietic stem cell transplantation has been well studied[1]. On the contrary, hematologic patients treated with high-dose chemotherapy and who underwent autologous stem cell transplantation (ASCT) were historically considered to have a low risk of CMV reactivation or end-organ disease. Previous studies on lymphoma and myeloma patients suggested an incidence of CMV reactivations of about 30%-40% when CMV determination was based on polymerase-chain reaction (PCR)/antigenemia prospective surveillance and of 1%-13% when determinations were performed only on the basis of clinical suspicion of infection, with a infection-mortality rate that ranged between 0% and 100%[2-11]. The guidelines of the European Conference on Infections in Leukemia (ECIL), published in 2008, consider the routine monitoring of CMV unnecessary in patients undergoing ASCT because of the low risk progression from infection to disease, with the exception of patients receiving CD34- selected grafts and prior treatment with Fludarabine, Cladribine or Alemtuzumab, considering that this setting of patients presented a profound alteration of T-cell-mediated immunity functional status[12]. However, the recent large use of immunotherapeutic drugs for the treatment of lymphomas and the introduction of proteasome inhibitors in the treatment of myeloma has determined an increase of viral infections also outside allogeneic transplantation setting, as for ASCT. In the last years, some studies have been published by our and others groups in order to better characterize the incidence of and the risk factors for CMV infection in ASCT of both in lymphoma and myeloma patients[13-17]. However, considering the low number of patients studied and to the multicenter nature of some previous studies (potential bias for the

Table 1 Patient characteristics at transplant *n* (%)

Median age (range)	**56 (18-72)**
Sex, M/F	**198/129**
Diagnosis	
Multiple myeloma	201 (61)
B-cell non-Hodgkin's lymphoma	80 (25)
Hodgkin's lymphoma	27 (8)
T-cell non-Hodgkin's lymphoma	19 (6)
CMV IgG seropositivity[1]	304 (93)
HBcIgG serositivity	46 (14)
HCVAb seropositivity[1]	5 (1.5)
Disease status	
Complete response	205 (63)
Partial response	114 (35)
Stable/progressive disease	8 (2)
Prior chemotherapy lines	
1	185 (57)
2	120 (37)
⩾ 3	22 (6)
Prior fludarabine treatment	5 (1.5)
Prior alemtuzumab treatment	0
Conditioning regimen	
BEAM or BEAM-like	126 (39)
MEL200/MEL100	201 (61)
Median $CD34^+$ infused cells $\times 10^6$/kg (range)	5.62 (2.36-28.48)

[1]Datum is missing in 2 patients. BEAM: Carmustine, Etoposide, Cytarabine, Melphalan; MEL200: Melphalan 200 mg/m^2; MEL100: Melphalan 100 mg/m^2; CMV: Cytomegalovirus.

heterogeneity of molecular virology laboratories and diagnostic strategies), data about this issue are not yet conclusive and needed to be validated. Based on these findings, the present study aimed to evaluate the risk factors for CMV symptomatic reactivation/end-organ disease and its impact in transplant-related mortality (TRM) in a large cohort of lymphoma and myeloma patients who underwent ASCT, under a unique and unchanged diagnostic strategy of this infection.

MATERIALS AND METHODS

Patients

A total of 327 consecutive non $CD34^+$ selected autografts performed from the Hematology and Stem Cell Transplantation Unit of Regina Elena National Cancer Institute of Rome (Italy) in the period comprised between January 2003 to January 2015, were reviewed. Over the 327 autografts, 201 were performed in patients with multiple myeloma, whereas the remaining 126 in patients affected by non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma. The patients who underwent an ASCT for an acute leukemia (n = 20) in the same period were excluded from this analysis. Patient characteristics at transplant are described in detail in Table 1. All patients were treated under a same anti-infectious and transfusional policy; in particular, all patients had received an antiviral prophylaxis with Valacyclovir and anti-Pneumocystiis prophylaxis with Cotrimoxazole given from the day of transplant until six months after and an antibacterial prophylaxis with Ciprofloxacin from the day of transplant until the resolution of severe neutropenia. All patients had signed an informed consent granting use of sensitive data for scientific purposes. The study has been approved by the institutional Ethical Committee.

Criteria for diagnosis of CMV symptomatic infection and end-organ disease

The criteria were based on published recommendations[12,18-20]. According to local policy and published guidelines[12], CMV DNAemia was determined only upon clinical suspicion of post-transplant reactivation, therefore no routine monitoring CMV strategy was adopted. Clinical suspicion criteria for check CMV DNAemia were defined as follows: presence of fever (temperature > 38 ℃) and overt clinical signs of bone marrow suppression, and in the absence of concomitant bacterial, viral (*i.e.,* HHV-6, EBV, parvovirus B19) or fungal co-infections (as demonstrated by clinical examination, thoracic computerized tomography, and repeated cultures from blood and urine). Bone marrow suppression was defined as a delay of neutrophilis and/or platelet recovery from ASCT (absence of complete neutrophilis and platelets recovery after 14 and 21 d from transplantation, respectively) or a drop in neutrophilis and/or platelet count after recovery (absolute count of neutrophilis or platelets < 1000/mcL or 100000/mcL, respectively, or a decrease of at least 30% of the counts in two consecutive determinations). CMV symptomatic infection was defined as a documented CMV DNAemia, confirmed by two consecutive determinations, in presence of clinical suspicion criteria of reactivation. CMV end-organ disease was defined by the presence of signs consistent with CMV infection, as determined by a combination of imaging and clinical and histopathological/molecular evaluations. In particular, CMV gastrointestinal disease was defined by the presence of a combination of clinical symptoms from the upper or lower gastrointestinal tract, findings of macroscopic mucosal lesions on endoscopy, and demonstration of the presence of CMV inclusion bodies in the tissue biopsy, further confirmed by positive immunohistochemical staining of CMV antigens in tissue sections of the gastrointestinal tract. CMV pneumonia was defined by the presence of clinical (hypoxemia) and radiological signs of interstitial pulmonary disease combined with the detection of high viral loads of CMV by quantitative PCR in bronchoalveolar lavage fluid confirmed by detection of CMV by direct immunostaining of alveolar cells[18,20]. Lung tissue biopsies to demonstrate the presence of CMV inclusion bodies in the tissue biopsy, were not performed considering the high risk of complications derived from a pulmonary biopsy in patients with a severe respiratory distress and a great hemorrhagic risk. In the presence of signs and symptoms of CMV reactivation, as above specified, an antiviral treatment was performed. The choice of antiviral agent to use for symptomatic reactivation treatment (Ganciclovir, Valganciclovir, Foscarnet sodium) was based on clinical features of the patients at the time

of reactivation.

Quantification of CMV DNA

Automated nucleic acid sample preparation systems NucliSENSeasyMAG® (BioMerieux, Durham, United States) has been used for DNA extraction from plasma, according to the manufacturer's instructions. Amplification for detection and quantification of viral DNA has been performed using commercially available real-time PCR assays (Affigene® CMV Trender diagnostic assay), according to the manufacturer's instructions (Cepheid AB, Bromma, Sweden) on a Mx3000P® System (Stratagene, La Jolla, CA, United States) until August 2013 then the analogous Geneproof CMV PCR kit (Czech Republic) on SLAN® Real-Time PCR Detection System (Shanghai Hongshi Medical Technology Co., Ltd). The limit of detection was 88 copies/mL in both kit.

Statistical analysis

Data were analyzed by Statistical Package of Social Sciences software (SPSS, version 17.0, Chicago, United States). Univariate analysis was performed in order to identify risk factors for clinically relevant CMV infection requiring specific treatment by using χ^2 test (Fisher or Pearson) and analysis of variance for categorical and quantitative variables, respectively. Two-sided *P*-values below 0.05 were considered to be statistically significant for the multivariate analysis. In case of two or more significant variables with reciprocal competitive effect, only the variable statistically more significant or clinically more relevant was included in the final model. Binary logistic regression model was used to analyze associations between significant baseline characteristics and the occurrence of CMV infection. Enter and remove limits were 0.05 and 0.1, respectively. TRM was estimated with the cumulative incidence method considering dead for relapse or other not transplant-related causes as competing risks. The curves of various subgroups were compared using Gray's test.

RESULTS

Clinical characteristics of patients at transplant are described in Table 1. The large majority of patients were seropositive for CMV IgG (304/327, 93%) and 46 (14%) were HBcIgG seropositive. Most of patients received un up-front ASCT (185/327, 57%) and 205 (63%) were transplanted in complete remission (CR). Median age at transplant was of 56 years (range: 18-72). Overall, 36 patients (11%) were treated with an antiviral therapy for a symptomatic CMV reactivation (n = 32) or an end-organ disease (n = 4). We observed 20 and 16 cases of CMV reactivation among lymphoma (16%) and myeloma patients (8%), respectively. The more relevant features of reactivation episodes are described in Table 2. Among cases of end-organ disease, 3 were diagnosed as interstitial pneumonia and one remaining case as hemorrhagic enteritis. We observed also three cases (8%) of extensive skin involvement by CMV infection, presenting as diffuse erythema not determined by others causes and promptly resolved after the begin of specific antiviral treatment. Median time from the transplant and the first detection of viral DNA in blood samples was of 33 d (range: 12-77). All cases of CMV reactivation were observed in IgG seropositive patients, with no documented cases of primary CMV infection. All patients were treated with a specific antiviral therapy (Table 2), with a global rate of hospitalization of 55%; four patients received intravenous immunoglobulins. The patients who experienced a symptomatic CMV reactivation presented a significant delay in neutrophilis and platelets recovery (P = 0.003 and P = 0.001, respectively). As for clinical outcome after antiviral treatment, 3 patients died, with a global mortality rate of 8%. However, we observe only one death directly related to CMV (respiratory distress caused by interstitial pneumonia), whereas in the others two cases, death was caused by gram negative septic shock. Figure 1 shows the cumulative incidence of 100-d TRM. As shown by the curves, TRM was significantly higher in patients who experienced a CMV reactivation (8.4% ± 4.7% *vs* 1.7% ± 0.8%; P = 0.047). A pre-transplant HBcIgG seropositivity, a diagnosis of T-cell NHL and an higher age at transplant were associated with the risk of post-transplant CMV reactivation, at univiariate analysis (P < 0.001, P = 0.042 and P = 0.004, respectively). All others baseline analyzed parameters, including sex, diagnosis (lymphoma *vs* myeloma), disease status at transplant, previous chemotherapy lines, conditioning regimes and median $CD34^+$ infused cells, resulted not statistically significant (data not shown). In multivariate analysis, a pre-transplant HBcIgG seropositivity (OR = 8.928, 95%CI: 1.991-33.321; P = 0.023) and a diagnosis of T-cell NHL (OR = 4.739, 95%CI: 1.511-11.112; P = 0.042) were independent risk factors for a post-transplant CMV reactivation.

DISCUSSION

CMV reactivation can be a relevant cause of morbidity following ASCT in adult lymphoma and myeloma patients. From our survey, 36 over 327 patients (11%) were treated for a post-transplant symptomatic CMV reactivation. Moreover, we observed 4 cases of end-organ disease (1%; 3 cases of interstitial pneumonia and 1 case of hemorrhagic enteritis). Cumulative incidence of TRM was significantly affected by the occurrence of a symptomatic CMV reactivation (5-fold risen, Figure 1), although only one death was directly attributable to CMV (respiratory distress caused by interstitial pneumonia), whereas the remaining two were caused by a gram negative bacterial co-infection, indirectly favored by the graft failure consequent to CMV reactivation. The global incidence of CMV reactivation and of end-organ disease observed in our study were substantially similar to our previously reports[14,15,17], but also to the others published studies in which it

Table 2 Clinical and laboratory features and outcome of cytomegalovirus reactivation episodes requiring specific antiviral treatment (36/327, 11%)

Clinical and laboratory features	No. of cases
Fever (temperature > 38 ℃ persistent at least 60 min)	36 (100%)
Signs of bone marrow suppression (delay of neutrophilis and/or platelet recovery or drop in neutrophilis and/or platelet count after recovery)	35 (97%)
DNAemia positivity (PCR assay)	36 (100%)
End-organ disease (according to published criteria)	4 (11%)
Interstitial pneumonia	3
Enteritis	1
Median number of CMV copies at first detection (range)[1]	895 (188-10120)
Median day from transplant at first detection (range)	33 (12-77)
Pre-transplant CMV IgG seropositivity	36 (100%)
Outcome	
Treatment[2]	
Ganciclovir	8
Foscarnet sodium	16
Valganciclovir	12
Immunoglobulins	4
Need of hospital admission	20 (55%)
Hematological recovery, median (range)[3]	
Neutrophilis > 500/mcL	14 (10-25)
Platelets > 20000/mcL	20 (11-88)
Alive	33 (92%)
Dead (48, 62, 89 d from transplant)	3 (8%)

[1]Limit of detection of PCR testing: 88 copies/mL; [2]Foscarnet sodium dosage: 60 mg/kg twice daily for 14 d, than 60 mg/kg per day for subsequent 5 d weekly for 2 wk); Ganciclovir dosage: 5 mg/kg twice daily for 14 d, than 5 mg/kg per day for subsequent 5 d weekly for 2 wk; Valganciclovir dosage: 900 mg twice daily for 14 d, than 900 mg/d for subsequent 5 d weekly for 2 wk; [3]The occurrence of a symptomatic CMV reactivation after ASCT, requiring antiviral treatment, leads to a delay in neutrophilis and platelets recovery (P = 0.003 and P = 0.001 respectively). ASCT: Autologous hematopoietic stem cell transplantation; CMV: Cytomegalovirus.

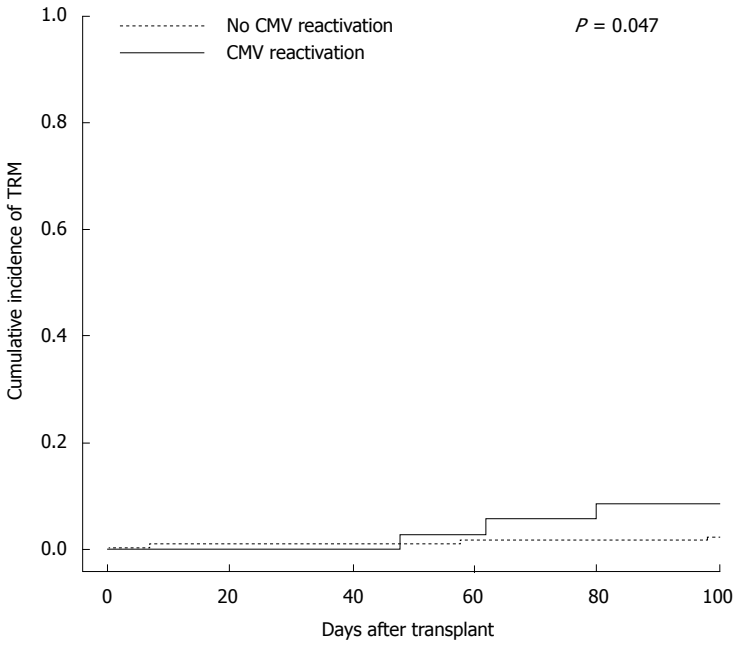

Figure 1 Cumulative incidence of 100-d transplant-related-mortality according to occurrence or not of a cytomegalovirus symptomatic reactivation/end-organ disease (8.4% ± 4.7% *vs* 1.7% ± 0.8%; *P* = 0.047). TRM: transplant-related-mortality; CMV: Cytomegalovirus.

has been used a same diagnostic strategy of CMV reactivation[5,7-10,21]. Data about the global incidence of CMV reactivation from our present study are particularly relevant because obtained in a single institution under a unique and unchanged strategy of diagnosis of this infection. Most of cases of CMV reactivation were

Table 3 Risk factors for the occurrence of cytomegalovirus symptomatic reactivation

Variables		Univariate analysis		Multivariate analysis	
		Occurrence of symptomatic infection or end-organ disease	*P*	OR (95%CI)	*P*
HBcIgG	Positive	16/46 (34%)	< 0.001	8.928 (1.991-33.321)	0.023
	Negative	20/281 (7%)			
Diagnosis	MM	16/201 (8%)	0.095	1.841 (1.058-5.633)	0.125
	Lymphoma	20/126 (16%)			
Diagnosis of T-cell NHL	Yes	6/19 (31%)	0.022	4.739 (1.511-11.112)	0.042
	No	30/308 (10%)			
Median age at transplant	Years (range)	60 (35-71) *vs* 52 (18-72)	0.004	2.922 (1.273-6.295)	0.088

MM: Multiple myeloma; NHL: Non-Hodgkin's lymphoma.

easily manageable, particularly in myeloma patients and about one third of cases (12/36, 33%; Table 2) were treated with oral Valganciclovir. However, considering that the occurrence of a symptomatic CMV reactivation had lead to a delay of neutrophilis and platelets recovery or to a graft failure, most of cases were treated with intravenous Foscarnet sodium, with a global rate of hospital re-admission of about 50% (Table 2). Our data confirm that lymphoma and myeloma patients who underwent an ASCT from CD34-non selected cells and not receiving Fludarabine and Alemtuzumab prior transplant are at low risk of CMV reactivation and a CMV end-organ disease is a rare event in this setting. However, it is an important cause of morbidity and, despite often easily manageable, CMV reactivation is also capable to affect TRM, as direct or indirect action. In our opinion, in this setting of patients, a prospective monitoring of PCR (surveillance strategy) is not recommended in all patients (according to ECIL guidelines[12]), but clinicians should be aware of this potentially severe complication, especially in the presence of post-transplant unexplained fever and drop in neutrophilis and platelets count. In this study, pre-transplant HBcIgG seropositivity is an independent factor able to predict the occurrence of a post-transplant CMV reactivation (Table 3). This datum, obtained in a larger number of lymphoma and myeloma patients and in a single-center setting, confirms our previous published results in lymphoma patients[14]. HBcIgG is a marker of occult hepatitis B virus (HBV) infection carrier. HBV positive patients could be considered as patients at risk for CMV reactivation[22,23]. The role of a HBV latent co-infection as independent factor for CMV reactivation observed in our study has a physiopathologic rationale, considering that interactions among some different viruses have been demonstrated to have a role in the pathogenesis of infections, through mechanisms of cross-permissiveness mediated by the immune system[14,24-26]. In fact, the mechanisms of virus-virus interaction is common and crucial to understanding pathogenesis of viral infections; we hypothesized that HBV is capable of favoring a CMV co-infection through direct interaction of viral molecules, but also trough acting on cell-mediate immune system[26]. However, contrasting data are recently obtained in allogeneic hematopoietic stem cell transplantation by Lin and collaborators, that suggest that the underlying HBV infection in donors or recipients before transplant does not increase the risk of CMV infection and end-organ disease[27]. Moreover, our data suggest for the first time that also a diagnosis of T-cell NHL seems to be an independent risk factor for post-transplant CMV reactivation in ASCT (Table 3). Although obtained on a small number of patients, also this datum is not surprising if we consider that CMV reactivation is associated with the presence of dysfunctional antigen-specific CD8+ cells[28] and that T-cell-mediated immunity plays a crucial role in the control of latent CMV infection. In this point of view, we could hypothesize that the impaired T-cell function observed in T-cell NHL is a favoring factor for post-transplant reactivation of CMV in autografted patients. In conclusion, from our study in adult lymphoma and myeloma patients undergoing ASCT, three issues may be addressed: (1) The incidence of CMV reactivation and end-organ disease are about of 11% and 1%, respectively. The occurrence of a CMV symptomatic reactivation is often easily manageable but is able to affect directly or indirectly the cumulative incidence of TRM (5-fold risen); (2) Our data confirm in a larger cohort of patients that a pre-transplant HBcIgG seropositivity is an independent risk factor for post-transplant CMV reactivation; and (3) With the caution due to limited number of patients, our data suggest for the first time that T-cell lymphoma patients could be also considered at high risk for post-transplant symptomatic CMV reactivation.

COMMENTS

Background

The introduction of novel immunosoppressive drugs in the treatment of hematologic malignancies had lead to an increase of interest for cytomegalovirus (CMV) infection also in setting different to allogeneic transplant. The authors reviewed 327 autografts performed in their institution with the aim to determine the incidence of and the risk factors for CMV symptomatic infection and end-organ disease after autologous stem cell transplantation.

Research frontiers

The search of risk factors of CMV reactivation in this setting of patients could

permit to individuate patients that could beneficiate of a surveillance diagnostic strategy of CMV reactivation, and also of a pre-emptive therapy.

Innovations and breakthroughs

This study validated our previous results and for the first time highlighted the role of a diagnosis of T-cell non-Hodgkin's lymphoma as risk factor for post-transplant CMV reactivation.

Applications

The findings found in this study could be used by clinicians to decide in which patients they could adopt a surveillance diagnostic strategy of CMV reactivation in autologous hematopoietic stem cell transplantation.

Terminology

Post-transplant CMV symptomatic infection is defined as a documented CMV DNAemia, confirmed by two consecutive determinations, in presence of clinical suspicion criteria of reactivation (*e.g.,* graft failure or drop in neutrophilis and platelets values, fever not explained).

Peer-review

The authors have performed a good study, the manuscript is interesting.

REFERENCES

1 **Ljungman P**, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. *Infect Dis Clin North Am* 2010; **24**: 319-337 [PMID: 20466273 DOI: 10.1016/j.idc.2010.01.008]

2 **Lin PC**, Lee MY, Lin JT, Hsiao LT, Chen PM, Chiou TJ. Virus reactivation in high-risk non-Hodgkin's lymphoma patients after autologous CD34+ -selected peripheral blood progenitor cell transplantation. *Int J Hematol* 2008; **87**: 434-439 [PMID: 18317882 DOI: 10.1007/s12185-008-0053-z]

3 **Rossini F**, Terruzzi E, Cammarota S, Morini F, Fumagalli M, Verga L, Elli E, Verga M, Miccolis I, Parma M, Pogliani EM. Cytomegalovirus infection after autologous stem cell transplantation: incidence and outcome in a group of patients undergoing a surveillance program. *Transpl Infect Dis* 2005; **7**: 122-125 [PMID: 16390400 DOI: 10.1111/j.1399-3062.2005.000111.x]

4 **Fassas AB**, Bolaños-Meade J, Buddharaju LN, Rapoport A, Cottler-Fox M, Chen T, Lovchik JC, Cross A, Tricot G. Cytomegalovirus infection and non-neutropenic fever after autologous stem cell transplantation: high rates of reactivation in patients with multiple myeloma and lymphoma. *Br J Haematol* 2001; **112**: 237-241 [PMID: 11167810 DOI: 10.1046/j.1365-2141.2001.02487.x]

5 **Boeckh M**, Stevens-Ayers T, Bowden RA. Cytomegalovirus pp65 antigenemia after autologous marrow and peripheral blood stem cell transplantation. *J Infect Dis* 1996; **174**: 907-912 [PMID: 8896489 DOI: 10.1093/infdis/174.5.907]

6 **Hebart H**, Schröder A, Löffler J, Klingebiel T, Martin H, Wassmann B, Gerneth F, Rabenau H, Jahn G, Kanz L, Müller CA, Einsele H. Cytomegalovirus monitoring by polymerase chain reaction of whole blood samples from patients undergoing autologous bone marrow or peripheral blood progenitor cell transplantation. *J Infect Dis* 1997; **175**: 1490-1493 [PMID: 9180191 DOI: 10.1086/516484]

7 **Bilgrami S**, Aslanzadeh J, Feingold JM, Bona RD, Clive J, Dorsky D, Edwards RL, Tutschka PJ. Cytomegalovirus viremia, viruria and disease after autologous peripheral blood stem cell transplantation: no need for surveillance. *Bone Marrow Transplant* 1999; **24**: 69-73 [PMID: 10435738 DOI: 10.1038/sj.bmt.1701827]

8 **Ng AP**, Worth L, Chen L, Seymour JF, Prince HM, Slavin M, Thursky K. Cytomegalovirus DNAemia and disease: incidence, natural history and management in settings other than allogeneic stem cell transplantation. *Haematologica* 2005; **90**: 1672-1679 [PMID: 16330442]

9 **Han XY**. Epidemiologic analysis of reactivated cytomegalovirus antigenemia in patients with cancer. *J Clin Microbiol* 2007; **45**: 1126-1132 [PMID: 17287334 DOI: 10.1128/JCM.01670-06]

10 **Holmberg LA**, Boeckh M, Hooper H, Leisenring W, Rowley S, Heimfeld S, Press O, Maloney DG, McSweeney P, Corey L, Maziarz RT, Appelbaum FR, Bensinger W. Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. *Blood* 1999; **94**: 4029-4035 [PMID: 10590046]

11 **Offidani M**, Corvatta L, Olivieri A, Rupoli S, Frayfer J, Mele A, Manso E, Montanari M, Centurioni R, Leoni P. Infectious complications after autologous peripheral blood progenitor cell transplantation followed by G-CSF. *Bone Marrow Transplant* 1999; **24**: 1079-1087 [PMID: 10578158 DOI: 10.1038/sj.bmt.1702033]

12 **Ljungman P**, de la Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P, Styczynski J, Ward K. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplant* 2008; **42**: 227-240 [PMID: 18587440 DOI: 10.1038/bmt.2008.162]

13 **Lee MY**, Chiou TJ, Hsiao LT, Yang MH, Lin PC, Poh SB, Yen CC, Liu JH, Teng HW, Chao TC, Wang WS, Chen PM. Rituximab therapy increased post-transplant cytomegalovirus complications in Non-Hodgkin's lymphoma patients receiving autologous hematopoietic stem cell transplantation. *Ann Hematol* 2008; **87**: 285-289 [PMID: 17943285 DOI: 10.1007/s00277-007-0397-0]

14 **Marchesi F**, Giannotti F, Avvisati G, Petti MC, Pimpinelli F, Paba P, Dessanti ML, Cerretti R, Tirindelli MC, Picardi A, D'Andrea M, Spadea A, Ensoli F, Perno CF, Mengarelli A, Arcese W. The potential role of pre-transplant HBcIgG seroposivity as predictor of clinically relevant cytomegalovirus infection in patients with lymphoma undergoing autologous hematopoietic stem cell transplantation: a study from the Rome Transplant Network. *Am J Hematol* 2012; **87**: 213-217 [PMID: 22076952 DOI: 10.1002/ajh.22214]

15 **Marchesi F**, Mengarelli A, Giannotti F, Tendas A, Anaclerico B, Porrini R, Picardi A, Cerchiara E, Dentamaro T, Chierichini A, Romeo A, Cudillo L, Montefusco E, Tirindelli MC, De Fabritiis P, Annino L, Petti MC, Monarca B, Arcese W, Avvisati G. High incidence of post-transplant cytomegalovirus reactivations in myeloma patients undergoing autologous stem cell transplantation after treatment with bortezomib-based regimens: a survey from the Rome transplant network. *Transpl Infect Dis* 2014; **16**: 158-164 [PMID: 24215479 DOI: 10.1111/tid.12162]

16 **Kim JH**, Goulston C, Sanders S, Lampas M, Zangari M, Tricot G, Hanson KE. Cytomegalovirus reactivation following autologous peripheral blood stem cell transplantation for multiple myeloma in the era of novel chemotherapeutics and tandem transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 1753-1758 [PMID: 22728249 DOI: 10.1016/j.bbmt.2012.06.008]

17 **Marchesi F**, Pimpinelli F, Dessanti ML, Gumenyuk S, Palombi F, Pisani F, Romano A, Spadea A, Maschio M, Ensoli F, Mengarelli A. Evaluation of risk of symptomatic cytomegalovirus reactivation in myeloma patients treated with tandem autologous stem cell transplantation and novel agents: a single-institution study. *Transpl Infect Dis* 2014; **16**: 1032-1038 [PMID: 25369809 DOI: 10.1111/tid.12309]

18 **Drew WL**. Laboratory diagnosis of cytomegalovirus infection and disease in immunocompromised patients. *Curr Opin Infect Dis* 2007; **20**: 408-411 [PMID: 17609601 DOI: 10.1097/QCO.0b013e32821f6010]

19 **Gor D**, Sabin C, Prentice HG, Vyas N, Man S, Griffiths PD, Emery VC. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. *Bone Marrow Transplant* 1998; **21**: 597-605 [PMID: 9543064 DOI: 10.1038/sj.bmt.1701139]

20 **Ljungman P**, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002; **34**: 1094-1097 [PMID: 11914998 DOI: 10.1086/339329]

21 **Boeckh M**, Gooley TA, Reusser P, Buckner CD, Bowden RA. Failure of high-dose acyclovir to prevent cytomegalovirus disease after autologous marrow transplantation. *J Infect Dis* 1995; **172**: 939-943 [PMID: 7561213 DOI: 10.1093/infdis/172.4.939]

22 **Bayram A**, Ozkur A, Erkilic S. Prevalence of human cytomegalovirus co-infection in patients with chronic viral hepatitis B and

C: a comparison of clinical and histological aspects. *J Clin Virol* 2009; **45**: 212-217 [PMID: 19497785 DOI: 10.1016/j.jcv.2009.05.009]

23 **Lian Y**, Wu W, Shi Y. [Preliminary study on relationship between different viral pathogenesis and disease prognosis in patients with severe viral hepatitis]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 1999; **13**: 355-357 [PMID: 12759976]

24 **Daar ES**, Lynn H, Donfield S, Gomperts E, O'Brien SJ, Hilgartner MW, Hoots WK, Chernoff D, Arkin S, Wong WY, Winkler CA. Hepatitis C virus load is associated with human immunodeficiency virus type 1 disease progression in hemophiliacs. *J Infect Dis* 2001; **183**: 589-595 [PMID: 11170984 DOI: 10.1086/318539]

25 **Cavanaugh VJ**, Guidotti LG, Chisari FV. Inhibition of hepatitis B virus replication during adenovirus and cytomegalovirus infections in transgenic mice. *J Virol* 1998; **72**: 2630-2637 [PMID: 9525579]

26 **DaPalma T**, Doonan BP, Trager NM, Kasman LM. A systematic approach to virus-virus interactions. *Virus Res* 2010; **149**: 1-9 [PMID: 20093154 DOI: 10.1016/j.virusres.2010.01.002]

27 **Liu YC**, Lu PL, Hsiao HH, Chang CS, Liu TC, Yang WC, Lin SF. Cytomegalovirus infection and disease after allogeneic hematopoietic stem cell transplantation: experience in a center with a high seroprevalence of both CMV and hepatitis B virus. *Ann Hematol* 2012; **91**: 587-595 [PMID: 21997849 DOI: 10.100/s00277-011-1351-8]

28 **Ozdemir E**, St John LS, Gillespie G, Rowland-Jones S, Champlin RE, Molldrem JJ, Komanduri KV. Cytomegalovirus reactivation following allogeneic stem cell transplantation is associated with the presence of dysfunctional antigen-specific CD8+ T cells. *Blood* 2002; **100**: 3690-3697 [PMID: 12393402 DOI: 10.1182/blood-2002-05-1387]

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ORIGINAL ARTICLE

Prospective Study

Weight trends in United States living kidney donors: Analysis of the UNOS database

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Abstract

AIM: To analyze the national trends associated with body mass index (BMI) and living kidney donation.

METHODS: Forty-seven thousand seven hundred and five adult living kidney donors as reported to the Organ Procurement and Transplantation Network from 1999 to 2011 were analyzed using their pre-donation BMI. Predictor variables of interest included age, gender, ethnicity, relationship, education status, and transplant region.

RESULTS: Sixteen thousand nine hundred and seventy-one of the living kidney donors were normal weight (35.6%); 19337 were overweight (40.5%); 9007 were mildly obese (18.9%); 1992 were moderate to morbidly obese (4.2%). Overweight and mildly obese kidney donors have increased through time by 12% and 20% every 5 years, respectively ($P < 0.05$). Donors 35-49

years of age, hispanic males or females and black females, those with high school diploma or general Education Degree, and biologically related or partner/spouses were more likely to be obese.

CONCLUSION: Over the past 13 years, the majority of living kidney donors have spanned the overweight to obese categories. Paralleling the national rise is an increase in overweight and mildly obese kidney donors. A fair number of moderate to morbidly obese living kidney donors are still allowed to donate.

Key words: Transplantation; Obesity; Donor; Kidney; Living

Core tip: The obesity epidemic is increasing. This study was conducted to analyze the national trends associated with body mass index (BMI) and living kidney donation using the United Network for Organ Sharing/Organ Procurement and Transplantation Network database in the United States. Forty-seven thousand seven hundred and five adult living kidney donors were analyzed according to BMI. Sixty-three point six percent of living kidney donors over the past thirteen years have spanned the overweight to obese categories. The increase in the overweight and mildly obese living kidney donors in our study parallels the national increase in obesity trends. A fair number of moderate to morbidly obese living kidney donors are still allowed to donate. Donors 35-49 years of age, hispanic males or females and black females, those with high school diploma or general Education Degree, and biologically related or partner/spouses were more likely to be obese. Care is advised when allowing donors in this BMI category to donate due to the uncertainty of the long term outcomes. Continued awareness and implementation of programs to limit the obesity crisis are needed.

Sachdeva M, Rosen LM, Varghese J, Fishbane S, Molmenti EP. Weight trends in United States living kidney donors: Analysis of the UNOS database. *World J Transplant* 2015; 5(3): 137-144 Available from: URL: http://www.wjgnet.com/2220-3230/full/v5/i3/137.htm DOI: http://dx.doi.org/10.5500/wjt.v5.i3.137

INTRODUCTION

The obesity epidemic has been increasing over the past three decades[1]. Measuring a height, weight, and calculating a body mass index (BMI) has been the recommended standard practice by the Organ Procurement and Transplantation Network (OPTN) as part of the physical evaluation of a potential living kidney donor[2]. According to OPTN guidelines, having a BMI greater than 35 kg/m^2 is considered a relative contraindication to be a living kidney donor[3]. Despite this, transplant centers across the United States use different criteria in determining donor exclusion based on BMI. Based on a 2007 United States Transplant Center Survey, twenty percent of the transplant centers that were surveyed excluded those with BMI greater than 40 kg/m^2, fifty two percent of United States kidney transplant centers excluded donors with BMI greater than 35 kg/m^2, ten percent of programs excluded those with BMI over 30 kg/m^2, twelve percent had no policy for exclusion, and six percent excluded based on BMI if they had other cardiovascular risks[4].

There is a shortage of living kidney donors. In 1999, per OPTN data, there were approximately 4728 living kidney donors. Although there has been an increase in the number of living kidney donors in the past 10 years, there is a downward trend since 2010. The number of living kidney donors went from 6278 to 5773 to 5619 to 5734 during 2010, 2011, 2012, and 2013 respectively[5]. These numbers of living kidney donor transplantations are not able to keep up with the increasing potential kidney recipients on the wait list which currently runs at approximately 103627[5].

Due to the shortage of living kidney donors, some transplant centers may be less stringent on the obesity criteria. However, the safety of potential donors must come first. Peri-operative and post-operative outcomes are concerns with obese kidney donors. Having a BMI greater than 35 kg/m^2 has been associated with slightly longer operative times and overall more peri-operative complications, such as wound complications[6]. In addition, long term outcomes for obese living kidney donors are still uncertain[7].

Due to the national shortage of living kidney donors and the parallel national increase in obesity, the primary aim of our study was to analyze the national temporal trends associated with BMI and living kidney donation over the past 13 years. In addition, we wanted to examine the association between live kidney donor BMIs and age, gender, race/ethnicity, relationship to the kidney transplant recipient, education status, transplant region, and year.

MATERIALS AND METHODS

Adult live kidney donors, over the age of 18 years in the United States from January 1st, 1999 to December 31st, 2011 were analyzed based on the United Network for Organ Sharing (UNOS)/OPTN standard transplant analysis and research files database. The study was performed with approval from the North Shore-LIJ Health System institutional review board.

The primary variable of interest was pre-donation BMI category. BMI was divided into five categories using the World Health Organization classification of obesity: Mildly thin was defined as BMI greater than or equal to 17 kg/m^2 and less than 18.5 kg/m^2. Normal weight was defined as a BMI greater than or equal to 18.5 kg/m^2 and less than 25 kg/m^2. Overweight was defined as a BMI greater than or equal to 25 kg/m^2 and less than 30

Table 1 Demographic Characteristics of United States living kidney donors from 1999-2011

Characteristic	(%)
Age	
18-34	31.7
35-49	44.5
50-64	22.5
⩾ 65	1.3
Gender	
Male	40.2
Female	59.8
Race/ethnicity	
Asian	3.7
Black	13.3
Hispanic	14.1
White	67.5
Other	1.4
BMI	
Mildly thin	0.8
Normal	35.6
Overweight	40.5
Mildly obese	18.9
Moderate/morbid obese	4.2
Living donor relationship	
Biological	61.5
Spouse/life partner	12.9
Non-biological	25.6
Education	
No HS diploma or GED	1.8
HS or GED	25.3
Attended college/technical school	22.1
Associate/bachelors degree	19.9
Graduate degree	8.1
Unknown	22.7
Region	
1	4.4
2	14.3
3	8.8
4	7.7
5	18.2
6	2.6
7	13.8
8	6.1
9	8.1
10	8.5
11	7.4

BMI: Body mass index; GED: General education degree; HS: High school.

kg/m^2. Mild obesity was defined as a BMI greater than or equal to 30 kg/m^2 and less than 35 kg/m^2. Moderate to morbid obesity was defined as a BMI greater than or equal to 35 kg/m^2. Multinomial logistic regression was used to model the outcome of donor BMI category: normal, overweight, mild obesity and moderate/morbid obesity. Due to the smaller number of subjects in the mildly thin category, it was excluded from the multinomial logistic regression analysis. Normal weight category was chosen as the reference.

Predictors of BMI included age category (18-34, 35-49, 50-65, ⩾ 65 years), gender, race/ethnicity (White, Black, Hispanic, Asian, Other), education (no high school diploma or general education degree (GED), high school diploma or GED (GED refers to testing that assures that the test taker is at high school level academic skills), attended college/technical school, associate/bachelor's degree, graduate degree), relationship to the organ recipient (non-biological, biological, partner/spouse), transplant region, and year. For demographics, descriptive statistics (mean, standard deviation, median, interquartile range-IQR, frequencies and percents) of demographic factors were used to describe the donors.

BMIs less than 17 kg/m^2 or over 45 kg/m^2 were considered implausible values and most likely to be erroneous entries, therefore, donors with BMI values outside of the 17 kg/m^2 to 45 kg/m^2 range were excluded. Donors less than 18 years of age or with a relationship status of paired exchange, deceased donor exchange or domino were excluded.

All analysis was conducted in SAS version 9.3 (SAS Institute, Inc., Cary, NC). Results were considered significant at $P < 0.05$.

RESULTS

There were a total of 53671 adult living donors who donated a kidney between 1999 and 2011. Five thousand seven hundred and sixty-four (10.7%) were removed due to missing BMI and 202 (0.4%) were removed for implausible values (see methods section). This resulted in 47705 adult live kidney donors who met the inclusion criteria. Characteristics of the live kidney donors are listed in Table 1. The average age was 40.69 ± 11.28 years. Females, whites, and biologically related donors comprised the majority of the live kidney donors. Few live donors were Asian. The average BMI was 26.87 ± 4.38 kg/m^2. Sixty-three point six percent of living kidney donors had BMI above 25 kg/m^2. 25.3% of donors had either a high school diploma/GED, and 22.1% had attended college/technical school.

Of the total donors who met the inclusion criteria, 398 were mildly thin (0.8%); 16971 were normal weight (35.6%); 19337 were overweight (40.5%); 9007 were in the mild obesity group (18.9%); 1992 in the moderate/morbidly obese group (4.2%). Figure 1 depicts the distribution of living kidney donors by BMI.

As depicted in Figure 2, over time, donors were less likely to be in the moderate/morbid BMI category as compared to the normal weight BMI category. More specifically, with each 5 year period, the odds of donors being in the moderate/morbid BMI category as compared to the normal weight BMI category decreased by 25% ($P < 0.05$). However, over time, donors were more likely to be in the mildly obese and overweight BMI categories as compared to the normal weight BMI category. More specifically, with each 5 year period, the odds of donors being in the mildly obese and overweight BMI categories as compared to normal weight BMI categories increased by 20% and 12%, respectively, $P < 0.05$.

Results from the multinomial logistic regression are summarized in Table 2. Live donor relationship ($P < 0.0001$), education ($P < 0.0001$), region ($P < 0.0001$)

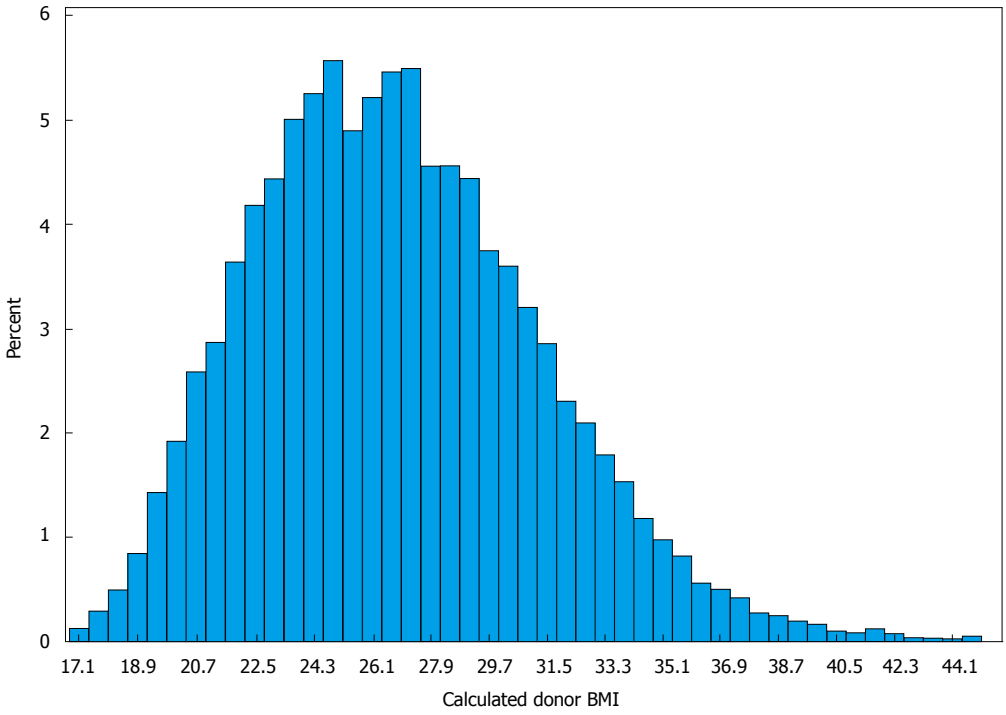

Figure 1 Distribution of living kidney donors by body mass index, 1999-2011. BMI: Body mass index.

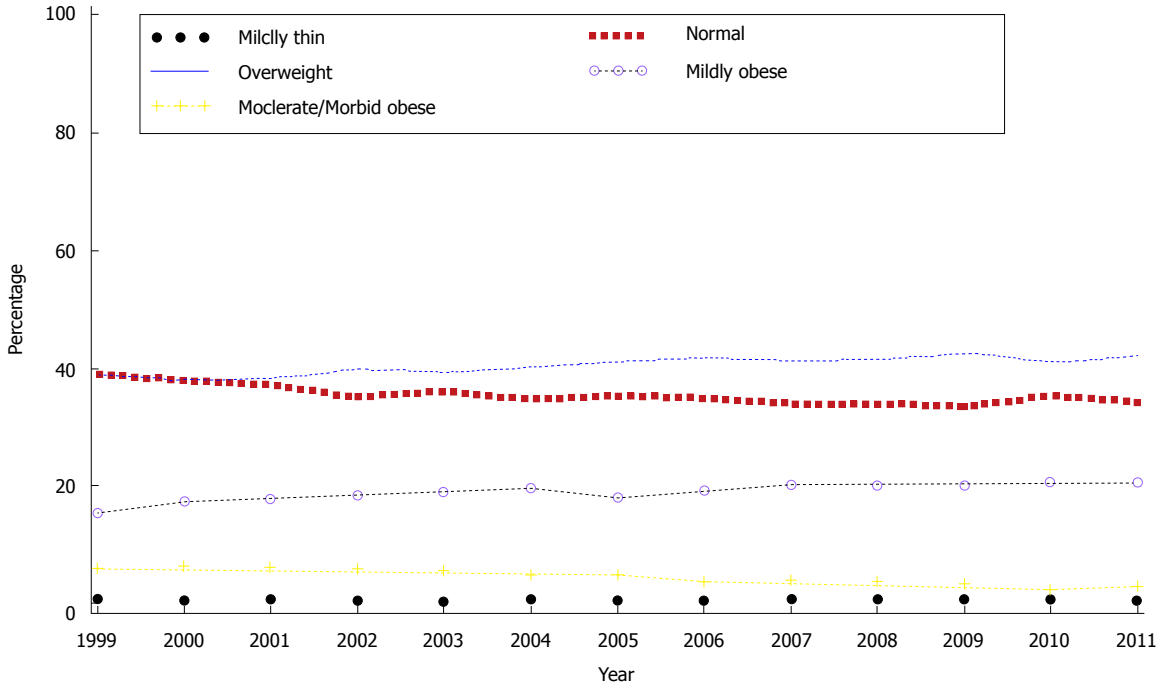

Figure 2 Percentage of living kidney donors by body mass index over time, 1999-2011.

and increasing year ($P < 0.0001$) were significantly associated with donor BMI category. Additionally, significant interactions were noted between donor age and gender ($P < 0.0001$), and ethnicity ($P < 0.0001$).

Age and gender

Male donors (35-49 years, and 50-64 years) were more likely to be obese and overweight than younger donors. Female donors 35-49 years of age had increased odds as compared to female donors 50-64 years of being moderately or morbidly obese (OR: 1.19, 95%CI: 1.02-1.40).

Race or ethnicity

Male and female Asian donors had decreased odds as compared to all other donors of being obese. Female

Table 2 Multinomial logistic regression models predicting moderate/morbid obesity (body mass index 35 or greater), mild obesity (body mass index 30 to less than 35) and overweight (body mass index 25 to less than 30) as compared to normal body mass index

Weight category *vs* normal weight	Moderate/morbid obese OR (95%CI)	Mild obese OR (95%CI)	Overweight OR (95%CI)
Relationship			
Biological	1.34 (1.19, 1.51)[a]	1.19 (1.11, 1.27)[a]	1.08 (1.03, 1.14)[a]
Partner/spouse	1.64 (1.39, 1.93)[a]	1.31 (1.19, 1.43)[a]	1.18 (1.09, 1.27)[a]
Non-biological	Ref.	Ref.	Ref.
Age x gender			
Males			
18-34 yr	Ref.	Ref.	Ref.
35-49 yr	1.65 (1.38, 1.97)[a]	1.84 (1.67, 2.02)[a]	1.82 (1.69, 1.97)[a]
50-64 yr	1.32 (1.04, 1.69)[a]	1.76 (1.56, 1.98)[a]	1.78 (1.61, 1.96)[a]
⩾ 65 yr	1.40 (0.66, 2.97)	1.47 (0.99, 2.16)	1.40 (1.03, 1.92)[a]
Females			
18-34 yr	Ref.	Ref.	Ref.
35-49 yr	1.03 (0.90, 1.17)	1.30 (1.20, 1.41)[a]	1.23 (1.15, 1.32)[a]
50-64 yr	0.86 (0.72, 1.02)	1.21 (1.10, 1.33)[a]	1.43 (1.33, 1.55)[a]
⩾ 65 yr	0.51 (0.25, 1.04)	0.73 (0.52, 1.04)	1.27 (1.01, 1.61)[a]
Ethnicity/race x gender			
Males			
Asian	0.31 (0.18, 0.55)[a]	0.22 (0.17, 0.30)[a]	0.49 (0.42, 0.58)[a]
Black	1.18 (0.95, 1.46)	0.99 (0.87, 1.12)	0.84 (0.76, 0.93)[a]
Hispanic	1.46 (1.16, 1.85)[a]	1.34 (1.18, 1.52)[a]	1.21 (1.09, 1.35)[a]
White	Ref.	Ref.	Ref.
Other	1.60 (0.85, 3.00)	1.23 (0.84, 1.79)	1.13 (0.82, 1.54)
Females			
Asian	0.15 (0.07, 0.30)[a]	0.33 (0.26, 0.42)[a]	0.54 (0.47, 0.63)[a]
Black	2.75 (2.36, 3.22)[a]	2.41 (2.17, 2.66)[a]	1.82 (1.66, 1.99)[a]
Hispanic	1.49 (1.24, 1.78)[a]	1.50 (1.35, 1.66)[a]	1.49 (1.37, 1.62)[a]
White	Ref.	Ref.	Ref.
Other	2.06 (1.34, 3.17)[a]	1.83 (1.40, 2.41)[a]	1.57 (1.25, 1.99)[a]
Education			
No HS diploma or GED	Ref.	Ref.	Ref.
HS or GED	2.03 (1.26, 3.28)[a]	1.08 (0.88, 1.32)	0.91 (0.77, 1.07)
Attended college/technical school	1.91 (1.18, 3.10)[a]	1.02 (0.83, 1.26)	0.93 (0.79, 1.10)
Associate/bachelors degree	1.40 (0.86, 2.28)	0.80 (0.65, 0.99)[a]	0.80 (0.67, 0.94)[a]
Graduate degree	1.04 (0.62, 1.73)	0.61 (0.49, 0.76)[a]	0.67 (0.56, 0.80)[a]
Unknown	1.84 (1.14, 2.98)[a]	1.03 (0.84, 1.27)	0.92 (0.77, 1.08)
Region			
1	1.18 (0.88, 1.57)	1.26 (1.08, 1.48)[a]	1.14 (1.01, 1.29)[a]
2	1.23 (0.99, 1.52)	1.09 (0.97, 1.23)	0.99 (0.90, 1.08)
3	1.08 (0.85, 1.37)	1.19 (1.05, 1.36)[a]	1.13 (1.02, 1.25)[a]
4	1.51 (1.19, 1.90)	1.46 (1.28, 1.66)[a]	1.15 (1.03, 1.28)[a]
5	0.78 (0.63, 0.97)[a]	0.99 (0.88, 1.11)	0.99 (0.91, 1.09)
6	0.85 (0.58, 1.26)	0.98 (0.81, 1.19)	1.07 (0.92, 1.24)
7	1.49 (1.21, 1.83)[a]	1.31 (1.17, 1.47)[a]	1.03 (0.94, 1.13)
8	0.71 (0.53, 0.95)[a]	0.92 (0.79, 1.06)	1.04 (0.93, 1.16)
9	Ref.	Ref.	Ref.
10	1.30 (1.03, 1.64)[a]	1.33 (1.17, 1.52)[a]	1.13 (1.02, 1.26)[a]
11	1.06 (0.83, 1.35)	1.23 (1.08, 1.41)[a]	1.11 (0.99, 1.23)
Year	0.75 (0.69, 0.80)[a]	1.20 (1.15, 1.25)[a]	1.12 (1.08, 1.15)[a]

[a]$P < 0.05$

BMI: Body mass index; GED: General education degree.

Blacks and Hispanics were more likely to be in the obese categories. Female Black donors had increased odds as compared to female Hispanic donors of being in higher BMI categories (moderate/morbid OR: 1.85, 95%CI: 1.50-2.29, mild OR: 1.61, 95%CI: 1.41-1.83). Male Hispanics were more likely to be obese as compared to male Black donors.

Relationship status

Biologically related donors and partner/spouse donors had increased odds as compared to non-biological donors of being obese as compared to normal weight donors. Partner/spouse donors had increased odds as compared to biological donors of being moderately or morbidly obese (OR: 1.22, 95%CI: 1.06, 1.40) and mildly obese (OR: 1.10. 95%CI: 1.01, 1.19).

Education

Donors with a High School (HS) diploma or GED had increased odds as compared to donors with an

associate/bachelor's degree and donors with a graduate degree of being moderately or morbidly obese (OR: 1.45, 95%CI: 1.25-1.68 and 1.96, 95%CI: 1.60-2.45, respectively) and mildly obese (OR: 1.35, 95%CI: 1.25-1.46 and 1.77, 95%CI: 1.58-1.97, respectively). Donors who attended college or technical school had increased odds as compared to donors with an associate or bachelor's degree and donors with a graduate degree of being moderately or morbidly obese (OR: 1.37, 95%CI: 1.17-1.59 and 1.85, 95%CI: 1.47-2.32, respectively) and mildly obese (OR: 1.28, 95%CI: 1.18-1.39 and 1.68, 95%CI: 1.50-1.87, respectively) as compared to normal weight.

Region

To help organ procurement, allocation, and transplantation, the United States is divided into 11 different UNOS regions. These regions correspond to some extent to the United States Census regions. There was a significant association between region and donor BMI. Region 9 had reduced odds as compared to other regions of having donors in higher BMI groups. Region 4 and Region 7 were more likely to have donors in the higher BMI groups.

DISCUSSION

Obesity is an increasing epidemic in the United States[8,9]. Sixty-three point six percent of living kidney donors in the past thirteen years have spanned the overweight to obese categories. The increase in the overweight and mildly obese living kidney donors in our study parallel the national increase in obesity trends.

Of concern is that obesity can be associated with an increased risk of hypertension, impaired fasting glucose, diabetes mellitus, dyslipidemia, metabolic syndrome, coronary artery disease, sleep apnea, and nonalcoholic fatty liver disease as well as an increased risk for certain cancers and indirectly through co-morbidities such as diabetes and hypertension, can lead to chronic kidney disease[10]. At five year follow up, Kramer *et al*[11] found that overweight and obese individuals had 20% and 40% risk of developing chronic kidney disease. Having a higher baseline BMI can serve as an independent risk factor for end stage kidney disease[12]. The long term effects of obesity on the solitary kidney of a kidney donor are still uncertain[7]. This risk factor increases the risk of developing other co-morbid conditions such as diabetes mellitus, hypertension, or even proteinuria which can together compromise the function of their solitary kidney. Since more than half of the living kidney donors in the past decade fall in the category of overweight or obese, concerns regarding post donation outcome should be taken into account. Obese donors should be counseled regarding their long term risk of developing the various aforementioned co-morbid conditions and regarding implementation of lifestyle modifications to try to decrease their risk. Due to the different BMI criteria of exclusion at different transplant centers, analysis revealed 1992 donors who were moderately to morbidly obese. Although a low net percentage of 4.2%, special concern and follow up should be dedicated to this subpopulation as they are likely to be of highest risk for subsequent co-morbidities. Short term outcomes of obese living kidney donors have shown increased wound related complications and longer operative times[6,13]. A recent meta-analysis found that operative duration, rise in serum creatinine, and conversion rate from laparoscopic donor nephrectomy to open procedure favored the lower BMI than higher BMI group[14]. Six months to one year follow up did not show any significant differences in renal function, creatinine levels, microalbuminuria, or hypertension when obese kidney donors were compared to their non-obese counterparts[6,15-17]. Still uncertain are the very long term outcomes in obese living kidney donors. At a mean of 11 year follow up, obese donors had an increased risk of developing hypertension and dyslipidemia, two important risk factors for coronary artery disease, however these were not found to be exacerbated by nephrectomy[17].

When a potential kidney donor comes for evaluation, certain donor demographics should be taken into consideration. For the obese kidney donor, especially in the 35-49 years old category cumulative donor health risk, may be increased throughout time. Biologically related and partner/spouses were more likely to be in the obese donor categories. This trend may be a reflection of the donors' willingness to do good for that close family member or loved one, blunting concern about themselves and their potential risks associated with their BMI.

Black and Hispanic females and Hispanic males were more likely than Whites and Asians to be obese donors. Our findings for males in regard to ethnicity deviated from the national trends. Among those greater than 20 years old, data from NHANES reveals that Non-Hispanic blacks have the highest age-adjusted rates of obesity (49.5%) compared with Mexican Americans (40.4%), Hispanics (39.1%), and non-Hispanic whites (34.3%)[18]. In our study, however, Hispanic males were more likely to be in the obese groups when compared to Blacks. Hispanic females were less likely to be in the obese groups when compared to Black females. This trend in the male Black population could be due to fact that Blacks were being excluded in the predonation period. Many Black obese donors may nevertheless end up being excluded because of obesity-related complications that have already developed prior to donation. In fact, in the pre-donation period, the majority of moderately to morbidly obese potential living kidney donors who were excluded were Black[19]. Of further concern is that Hispanics and Blacks are at highest risk for hypertension and kidney disease. Being of Hispanic ethnicity, increased the risk of end stage renal disease and progression of end stage renal disease, partially explained by higher prevalence of diabetes in this group[20]. Blacks as well have a higher prevalence of

ESRD[17,21]. Informed consent and risk stratification of these donors in the predonation evaluation period is imperative.

The UNOS/OPTN database is the only national database for living kidney donors. As with all databases, there are limitations. There can be under reporting and missing donor data as well as inaccurately entered data. Ten point seven percent of the total donors had missing BMI and less than 1% had implausible data entries as discussed in the methods section. A strength is the large number of living kidney donors and the diversity of donors in the database. Another limitation of this study is that the database does not capture how many potential living kidney donors were excluded due to obesity or obesity related complications in the predonation evaluation period, as more than fifty percent of United States transplant centers are excluding those with BMI greater than 35 kg/m^2. Prior studies have shown that obesity is serving as a potential barrier to kidney donation[19,22]. Although we do see an upward trend in the overweight and mildly obese paralleling the national trend, knowing this predonation information would allow us to demonstrate an accurate trend of kidney donors in this higher BMI category.

The obesity epidemic is affecting the living kidney donor population. Paralleling the national rise, there is an increase in the overweight and mildly obese kidney donors. In addition, there still remains a small number of moderate to morbidly obese donors who are allowed to donate. Care is advised when allowing donors in this BMI category to donate due to the uncertainty of the long term outcomes. On a national level, continued awareness and implementation of programs to limit the obesity crisis are needed.

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COMMENTS

Background

The obesity epidemic has been increasing over the past three decades. Transplant centers across the United States use different criteria in determining donor exclusion based on body mass index (BMI). There is a national shortage of living kidney donors. Due to the shortage of living kidney donors, some transplant centers may be less stringent on the obesity criteria. However, the safety of potential donors must come first. Long term outcomes for obese living kidney donors are still uncertain. The primary aim of the study was to analyze the national temporal trends associated with BMI and living kidney donation over the past 13 years. In addition, the authors wanted to examine the association between live kidney donor BMIs and age, gender, race/ethnicity, relationship to the kidney transplant recipient, education status, transplant region, and year.

Research frontiers

This study allows us to see the temporal trend of BMI and living kidney donation. It highlights certain donor characteristics which should be taken into account when a potential kidney donor is evaluated. Since most of the living kidney donors fall in the overweight or obese categories, hence contributing to the majority of the living kidney donor encounters, and due to uncertain long term outcomes in the obese living kidney donor, this study highlights the importance of discussing all possible long term co-morbidities and complications associated with obesity during an initial donor evaluation.

Innovations and breakthroughs

By analyzing the temporal trend of BMI and living kidney donation, the authors were able to determine where the authors stand in relationship to the obesity epidemic. The authors found that 63.6% of living kidney donors over the past thirteen years have spanned the overweight to obese categories. The increase in the overweight and mildly obese living kidney donors in the study parallels the national increase in obesity trends. Seeing that more than half of the living kidney donors fall in the overweight to obese categories, something needs to be done to address the obesity epidemic. In addition, there were 1992 in the moderate/morbidly obese group who were allowed to donate. Although a low net percentage of 4.2%, special concern and follow up should be dedicated to this subpopulation as they are likely to be of highest risk for subsequent co-morbidities. In addition, the authors found that donors 35-49 years of age, hispanic males or females and black females, those with high school diploma or general education degree, and biologically related or partner/spouses were more likely to be obese. These certain donor demographics should be taken into account when a potential kidney donor comes in for evaluation.

Applications

The authors see that more of the donors are overweight and obese and still there are a minority of kidney donors who are moderate to morbidly obese. Caution should be taken when allowing these donors to donate due to uncertain long term kidney donation outcomes in this subpopulation. The first priority in donor evaluation should be to assess safety and to discuss all potential long term comorbidities and complications with this subpopulation. In addition, a call for national and international programs is needed to stop the obesity epidemic.

Peer-review

This is a well written paper.

REFERENCES

1 **Barry CL**, Gollust SE, Niederdeppe J. Are Americans ready to solve the weight of the nation? *N Engl J Med* 2012; **367**: 389-391 [PMID: 22853011 DOI: 10.1056/NEJMp1206519]
2 OPTN Policies and Bylaws For Living Kidney Donation. 2015. Available from: URL: http://optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/policy_172.pdf
3 OPTN/UNOS Living Kidney Donor Report. 2015. Available from: URL: http://optn.transplant.hrsa.gov/CommitteeReports/board_main_LivingDonorCommittee_11_14_2012_11_34.pdf
4 **Mandelbrot DA**, Pavlakis M, Danovitch GM, Johnson SR, Karp SJ, Khwaja K, Hanto DW, Rodrigue JR. The medical evaluation of living kidney donors: a survey of US transplant centers. *Am J Transplant* 2007; **7**: 2333-2343 [PMID: 17845567 DOI: 10.1111/j.1600-6143.2007.01932.x]
5 Organ Procurement and Transplantation Network Annual Report. 2015. Available from: URL: http: //optn.transplant.hrsa.gov/latestData/rptData.asp
6 **Heimbach JK**, Taler SJ, Prieto M, Cosio FG, Textor SC, Kudva YC, Chow GK, Ishitani MB, Larson TS, Stegall MD. Obesity in living kidney donors: clinical characteristics and outcomes in the era of laparoscopic donor nephrectomy. *Am J Transplant* 2005; **5**: 1057-1064 [PMID: 15816886 DOI: 10.1111/j.1600-6143.2005.00791.x]
7 **Nogueira JM**, Weir MR, Jacobs S, Breault D, Klassen D, Evans DA, Bartlett ST, Cooper M. A study of renal outcomes in obese living kidney donors. *Transplantation* 2010; **90**: 993-999 [PMID:

20844468 DOI: 10.1097/TP.0b013e3181f6a058]
8 **Flegal KM**, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; **288**: 1723-1727 [PMID: 12365955 DOI: 10.1001/jama.288.14.1723]
9 **Flegal KM**, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 2010; **303**: 235-241 [PMID: 20071471 DOI: 10.1001/jama.2009.2014]
10 **Dietz WH**. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics* 1998; **101**: 518-525 [PMID: 12224658]
11 **Kramer H**, Luke A, Bidani A, Cao G, Cooper R, McGee D. Obesity and prevalent and incident CKD: the Hypertension Detection and Follow-Up Program. *Am J Kidney Dis* 2005; **46**: 587-594 [PMID: 16183412 DOI: 10.1053/j.ajkd.2005.06.007]
12 **Hsu CY**, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. *Ann Intern Med* 2006; **144**: 21-28 [PMID: 16389251 DOI: 10.7326/0003-4819-144-1-200601030-00006]
13 **Pesavento TE**, Henry ML, Falkenhain ME, Cosio FG, Bumgardner GL, Elkhammas EA, Pelletier RP, Ferguson RM. Obese living kidney donors: short-term results and possible implications. *Transplantation* 1999; **68**: 1491-1496 [PMID: 10589945 DOI: 10.1097/00007890-199911270-00011]
14 **Lafranca JA**, Hagen SM, Dols LF, Arends LR, Weimar W, Ijzermans JN, Dor FJ. Systematic review and meta-analysis of the relation between body mass index and short-term donor outcome of laparoscopic donor nephrectomy. *Kidney Int* 2013; **83**: 931-939 [PMID: 23344469 DOI: 10.1038/ki.2012.485]
15 **Reese PP**, Feldman HI, Asch DA, Thomasson A, Shults J, Bloom RD. Short-term outcomes for obese live kidney donors and their recipients. *Transplantation* 2009; **88**: 662-671 [PMID: 19741463 DOI: 10.1097/TP.0b013e3181b27a17]
16 **Aggarwal N**, Porter AC, Tang IY, Becker BN, Akkina SK. Creatinine-based estimations of kidney function are unreliable in obese kidney donors. *J Transplant* 2012; **2012**: 872894 [PMID: 22315657 DOI: 10.1155/2012/872894]
17 **Tavakol MM**, Vincenti FG, Assadi H, Frederick MJ, Tomlanovich SJ, Roberts JP, Posselt AM. Long-term renal function and cardiovascular disease risk in obese kidney donors. *Clin J Am Soc Nephrol* 2009; **4**: 1230-1238 [PMID: 19443625 DOI: 10.2215/CJN.01350209]
18 **Flegal KM**, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 2012; **307**: 491-497 [PMID: 22253363 DOI: 10.1001/jama.2012.39]
19 **Sachdeva M**, Sunday S, Israel E, Varghese J, Rosen L, Bhaskaran M, Molmenti EP, Mattana J. Obesity as a barrier to living kidney donation: a center-based analysis. *Clin Transplant* 2013; **27**: 882-887 [PMID: 24102846 DOI: 10.1111/ctr.12246]
20 **Peralta CA**, Shlipak MG, Fan D, Ordoñez J, Lash JP, Chertow GM, Go AS. Risks for end-stage renal disease, cardiovascular events, and death in Hispanic versus non-Hispanic white adults with chronic kidney disease. *J Am Soc Nephrol* 2006; **17**: 2892-2899 [PMID: 16959827 DOI: 10.1681/ASN.2005101122]
21 **Hsu CY**, Lin F, Vittinghoff E, Shlipak MG. Racial differences in the progression from chronic renal insufficiency to end-stage renal disease in the United States. *J Am Soc Nephrol* 2003; **14**: 2902-2907 [PMID: 14569100 DOI: 10.1097/01.ASN.0000091586.46532.B4]
22 **Stewart Z**. Obesity Is a Major Barrier to Increasing Living Kidney Donation in the United States. *ATC* 2013: Abstract 70

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