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STUDY OF THE RELATION BETWEEN RECEIVING ANTI-THYMOCYTE GLOBULIN AND MORBIDITY & MORTALITY IN HAEMATOPOIETIC TRANSPLANTED PATIENTS

By

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ABSTRACT

Background: Since the first successful bone marrow transplant in 1959, thousands of patients with lethal diseases such as severe leukemia, a plastic anemia, and inherited immune deficiencies have been successfully treated with hematopoietic stem cells (HSC). But for all the success stories, transplant physicians seeking to make HSC safer and more widely available continue to grapple with the problems of a limited donor pool, graft rejection, and graft vs.-host disease (GVHD). Bone marrow for many years was virtually the only source of HSC-self-renewing, unspecialized cells that give rise to all of the hematologic and immunologic cells-but transplant physicians increasingly are making use of stem cells collected from peripheral blood or the umbilical cord.

Objective: The aim of this work is to study the effect of receiving ATG in allo-HSCT (Hematopoietic Stem Cell Transplantation) on morbidity as GVHD; type of infections; frequency& mortality and survival rate

Patients and Methods: This was an observational cohort retrospective & prospective study, done on 200 patients collected from bone marrow transplantation units at Ain-Shams University& International Medical Center, Cairo-Egypt from August, 2019August, 2021. All participants underwent to clinical examination, laboratory findings, flowcytometry & cytogenetic analysis and all required investigations after explanation of the procedure to the patients and getting a formal consent. This study was approved by the ethical committee of faculty of medicine, Cairo, Al-Azhar University.

Results: there was a statistical difference (P- value <0.05) in the incidence and severity of GVHD between the two studied groups which was lower in the ATG arm than non ATG. Also, ATG was associated with a higher rate of infection which was also statistically significant (P-value <0.05) between the studied gropes. As regard survival rates ATG was associated with a higher survival rate which was statistically significant (P-value <0.05).

Conclusion: ATG was associated with a lower incidence rate and severity of GVHD whether acute or chronic but, on the contrary was associated with increased risk of infections.

Key Words: HSCT, ATG& GVHD.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) cures a wide range of hematological disorders. Allo-HCT with a myeloablative conditioning (MAC) regimen remains the treatment of choice for high-risk acute myeloid leukemia (AML) *(Scott et al., 2017)*.

Graft-versus-host disease (GvHD) is one of the most important factors limiting the success of allogeneic hematopoietic stem cell transplantation (HSCT) *(Wingardet al., 2011)* because it negatively affects both the duration and quality of life of patients after transplant *(Pidalaet al., 2011)*.

The most widely used strategy for GVHD prevention, at least in Europe *(Ruutu et al., 2012)*, is the addition of rabbit antilymphocyte globulin to the standard prophylaxis with a calcineurin inhibitor and either methotrexate or mycophenolate mofetil. There are several formulations of polyclonal antilymphocyte sera available in different countries, generated in animals (rabbits, horses, and pigs) by inoculation of human thymocytes or human cell line. Available rabbit polyclonal antilymphocyte sera are the antithymocyte globulin (ATG) (Sanofi Genzyme, Cambridge MA), derived from rabbit vaccination with human thymocytes, and the anti-T lymphocyte globulin (ATLG) (Neovii, Rapperswil, Switzerland, formerly ATG Fresenius®), derived from the human Jurkat T-cell line. Horse ATG (hATG, ATGAM®, Pfizer Inc NY, US) is used as first-line therapy of moderate–severe aplastic anemia *(Scheinberg et al., 2001)* as well as GvHD prophylaxis *(Kekre et al., 2017)*. Finally, porcine ATG is also used for the treatment of severe aplastic anemia especially in China and India *(chenM et al., 2016)* and, less frequently, in the setting of HSCT *(Chen X et al., 2016)*. Here we restrict the consensus to rabbit sera because of their prevalent use as drugs for GvHD prevention.

Data regarding the use of rabbit polyclonal serum in patients undergoing allogeneic HSCT indicate that both ATG and ATLG significantly reduce the incidence of GvHD but their administration is associated with delayed immune reconstitution *(Servais et al., 2015)*. As a consequence, they lead potentially to an increased risk of infections and of leukemia relapse. Despite the most recent meta-analysis did not show any significant benefit of ATG/ATLG on post-transplant survival *(Kumar et al., 2018)* data from different studies are still discordant because of different formulations, doses, and timing used as well as the heterogeneity of the studied populations, the hematopoietic stem cell sources, and the intensity of the conditioning regimens.

PATIENTS AND METHODS

This is an observational cohort prospective & retrospective study, done on two hundred allogeneic transplanted patients admitted in bone marrow transplantation units at Ain-Shams University Hospital (Al-Demerdash) & International Medical Center (IMC), Cairo, Egypt during the period from August, 2019 to August, 2021.

All data of patients admitted in both units collected and analyzed, starting from history taking, physical examination,

required investigations, before transplantation. Also type of remission, conditioning program and post-transplant follow up was done after explaining all steps to the patients and getting informed consent according to ethical committee.

Patients were divided into two groups:

Group (A): Patients received ATG.

Group (B): Patients did not receive ATG.

Exclusion criteria: Patients with previous history of CMV or previous allo-haemopoietic stem cell transplantation and/ or received ATG before.

Statistical analyses: Data were analyzed using Statistical Package for the Social Science (SPSS) version 24. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

Independent-samples t-test of significance was used when comparing between two means.

Mann Whitney U test was used when comparing between two means (for abnormal distributed data).

Chi-square test was used when comparing between non-parametric data.

A one-way analysis of variance (ANOVA) was used on comparing between more than two means.

Post Hoc test was used for multiple comparisons between different variables.

RESULTS

Regarding description of demographic data, the study was done on two hundred patients divided in two groups classified according to receiving ATG or not, all patients in the ATG arm was below 16 years old while, patients in the no ATG arm was above 16 years old **(Table 1)**.

As regard to cytogenetics of the underlying disease, high risk cytogenetics were higher in the non ATG group compared to the ATG group which was secondary to the high variations in age in that group as represented in table 2 while, patients with not available cytogenetics were higher in the ATG group because most of those patient's data was collected from their files **(Table 2)**.

Table (1): Comparison between studied groups as regard age and sex

		ATG (N = 100)		Non ATG (N = 100)	
Age	2–16 years	100	100%	39	39%
	25 - 35 years	0	0%	38	38%
	35 - 45 years	0	0%	18	18%
	45 - 55 years	0	0%	4	4%
	55 - 65 years	0	0%	1	1%
Sex	Male	47	47%	60	60%
	Female	53	53%	40	40%

Table (2): Comparison between studied groups as regard cytogenetics

		ATG (N = 100)		Non ATG (N = 100)		X2	P-value
Cytogenetics	Not available	83	83%	3	3%	133.7	< 0.001 HS
	Low risk	0	0%	25	25%		
	Intermediate risk	8	8%	27	27%		
	High risk	9	9%	45	45%		

X2: Chi-square test. HS: p-value < 0.001 is considered highly significant.
NS: p-value > 0.05 is considered non-significant.

Results as regard incidence and severity of acute GVHD showed statistical difference between the two studied groups which was lower in the ATG group than non ATG (p-value 0.014 & 0.0141) respectively **(Table 3)**. Also, for comparison between the studied groups as regard incidence & severity of chronic GVHD, there was statistical difference between them, that was lower in the ATG group compared to non ATG (P-value 0.001& 0.041) respectively **(Table 4)**.

Table (3): Comparison between studied groups as regard Acute GVHD

		ATG (N = 100)		Non ATG (N = 100)		X2	P-value
Acute GVHD	Negative	77	77%	61	61%	5.9	0.014 S
	Positive	23	23%	39	39%		
Acute GVHD grade	Grade I	9	39.1%	9	23.1%	8.2	0.041 S
	Grade II	6	26.1%	6	15.4%		
	Grade III	7	30.4%	10	25.6%		
	Grade IV	1	4.3%	14	35.9%		

X2: Chi-square test, S: p-value < 0.05 is considered significant.

Table (4): Comparison between studied groups as regard chronic GVHD

		ATG (N = 100)		Non ATG (N = 100)		X2	P-value
Chronic GVHD	Negative	65	65%	29	29%	26.01	<0.001HS
	Positive	35	35%	71	71%		
Chronic GVHD grade	Grade I	7	20%	20	28.2%	8.2	0.041 S
	Grade II	19	54.3%	18	25.4%		
	Grade III	5	14.3%	18	25.4%		
	Grade IV	4	11.4%	15	21.1%		

X2: Chi-square test, S: p-value < 0.05 is considered significant.

Table (5): Comparison between studied groups as regard infections post-transplant

		ATG (N = 100)		Non ATG (N = 100)		X2	P-value
Bacterial	Negative	40	40%	56	56%	5.12	0.023 S
	Positive	60	60%	44	44%		
Viral	Negative	18	18%	31	31%	4.56	0.032 S
	Positive	82	82%	69	69%		
Fungal	Negative	78	78%	80	80%	5.7	0.016 S
	Positive	29	29%	15	15%		

X2: Chi-square test, S: p-value < 0.05 is considered significant.

When the impact of ATG on rate & type of infection was compared, there was a higher incidence of infection bacterial, viral or fungal with statistically significant difference which was higher in ATG group than non ATG group (p-value 0.023, 0.032, 0.016) respectively **(Table 5)**. In spite of that viral infection has the higher burden but, when doing univariate analysis there was no statistical difference (P-value 0.109) **(Table 6)**.

Table (6): Comparison between studied groups as regard type of post- transplant viral infection

		ATG (N = 82)		Non ATG (N = 69)		X2	P-value
Viral	CMV	25	30.5%	16	23.2%	6.04	0.109 NS
	EBV	28	34.1%	15	21.7%		
	HZV	17	20.7%	22	31.9%		
	HSV	12	14.6%	16	23.2%		

X2: Chi-square test, NS: p-value > 0.05 is considered non-significant.

When we compared between the two groups for overall survival (OS) & disease-free survival (DFS) there was better survival rates for ATG group with statistical difference (P-value 0.029 & 0.019) respectively. Also, there was a lower incidence of relapse in ATG group with a statistical difference (P-value 0.044) as shown in **Table 7**.

Table (7): Comparison between studied groups as regard overall survival till the end of the study

		ATG (N = 100)		Non ATG (N = 100)		Test	P-value
Overall survival by months	Median	22.5		8		MW = 3933.5	0.029 S
	IQR	6 – 61		2.5 – 62			
relapse free survival by months	Median	22		6		MW = 4041.5	0.019 S
	IQR	6 – 57		2.12 – 60			
Relapse	Negative	90	90%	97	97%	X2 = 4.03	0.044 S
	Positive	10	10%	3	3%		

X2: Chi-square test, S: p-value < 0.05 is considered significant.
MW: Mann Whitney U test.

DISCUSSION

The impact of ATG use following allo-hematopoietic stem cell transplantation (HSCT) has been poorly explored in Egypt especially in adults in spite of, its good role in reducing the incidence of GVHD which is a major obstacle following HSCT as mentioned before then, we conducted this study as a beginning for clarifying the outcome of using ATG in conditioning regimens in our country, Egypt.

Our results came in agreement with *Arnon et al., 2019* who found a low incidence rate of aGVHD & cGVHD in the ATG group than in non ATG group also, the result came in agreement with *Finke et al., 2009* study who found the same results between the studied groups.

When studying the rate of total infection, it was higher in the ATG group than non ATG group that came in agreement with Lee et al., 2014, a non-randomized comparison between 60 mg/kg vs.30 mg/kg of ATLG, in patients with hematological malignancies receiving a MAC regimen, showed that higher doses were associated with an inferior outcome because of greater fatal infections, while aGvHD, cGvHD, and relapse were similar in the two groups. Although, most of our patients were receiving ATG not ATLG as in the above-mentioned study, but this can be considered a supporting point confirming that ATG serum broadly has a benefit for GVHD reduction but, might increase infection when used in high doses.

Also, for the higher incidence of infection with ATG use, our results came in agreement with *Romberger et al., 2014* that found a trend toward increased infectious mortality with 10mg/kg compared with 4-8mg/kg.

When comparing the type of infection between the two groups viral infection has the upper hand as about 80% of patients got viral infection either a new infection or reactivation especially for CMV& EBV which came in agreement with *Hoegh et al., 2013* also, with *Romberger et al., 2014* study who found there was a trend toward increased percentage of patients with > 1 viral infection among patient treated with 10 versus 4 to 8 mg/kg ATG.

Our results came in disagreement with *Soiffer RJ., 2017*, they found in a recent prospective double-blind phase 3 study of ATG vs placebo in patients with myelodysplastic syndrome or acute leukemia who underwent allo-HCT from a matched unrelated donor (aMUD) following myelo-ablative conditioning (MAC) regimens inferior PFS and OS in the ATG arm, especially in the TBI cohort which may be explained by the difference in samples which was higher in number and more homogenous than our sample, which conducted on almost two different age and disease groups. However, several other open label randomized trials *Finke et al., 2017* & *Kroger et al., 2016* have reported a benefit of ATG with a lower incidence of cGVHD without negatively impacting PFS or OS as came in our study.

CONCLUSION

In summary, incorporation of ATG-based in vivo T-cell depletion (TCD) in conditioning regimens for allo-HCT resulted in lower GVHD rates and improved non relapse mortality (NRM),

without increasing the disease relapse rate. Although this is a registry-based observational study, it is the first analysis of its kind and it demands a prospective validation of the role of ATG in allo-HCT conditioning. Future studies still needed to highlight the dose & timing of ATG administration for better outcome.

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دراسة العلاقة بين العلاج بمضاد الجلوبولين التيموسي و معدل المضاعفات والوفاة عند مرضي زرع النخاع

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خلفيـــة البحـــث: تهـــدف عمليـــة زرع النخـــاع فـــي المقـــام الأول فـــي الحصـــول علـــي المنفعـــة مـــن زرع خلايـــا جزعيـــة صـــحية فـــي جســـد المـــريض بـــدلا مـــن الخلايـــا الغيـــر صـــحية -بعـــد الـــتخلص منهـــا مـــن خـــلال جرعـــات مـــن الأدويـــة اللكيماويـــة التـــي تعطـــي للمـــريض خـــلال برنـــامج التحضـــير لعمليـــة زرع النخـــاع العظمـــي- دون تغلـــب هـــذه الخلايـــا المنزرعـــة علـــي جســـد المـــريض وحـــدوث مايســـمي ب داء مهاجمـــة الخلايـــا المزروعة لجسد المريض.

يعمـــل مضـــاد الجلوبـــولين المنـــاعي علـــي تقليـــل أو الـــتخلص مـــن الخلايـــا التائيـــة المناعيـــة للعائـــل وكـــذلك مـــن الخلايـــا المزروعـــة ممـــا يقلـــل مـــن حـــدوث داء مهاجمـــة الخلايـــا لجســـد المـــريض، لكـــن قـــد يزيـــد مـــن حـــدوث تغلـــب للميكروبـــات الضـــارة فـــي جســـم المـــريض ممـــا يـــؤدي لحـــدوث الأمـــراض، لـــذا يجـــب موازنـــة المنفعـــة والمضـــرة قبـــل اعطائـــه وكـــذلك حســـاب الجرعـــات المناســـبة طبقـــا للأبحـــاث العلميـــة والتوصـــيات الطبية.

الهـــدف مـــن البحـــث: دراســـة العلاقـــة بـــين اعطـــاء مضـــاد الجلوبـــولين المنـــاعي ومعـــدل الاصابة والوفاة عند المرضي ونسبة حدوث داء مهاجمة الخلايا لجسد المريض.

المرضـــى وطـــرق البحـــث: هـــذه دراســـة جماعيـــة أجريـــت علـــى مـــائتي حالـــة مـــن مرضـــي زرع النخـــاع مـــن متبـــرع، تـــم علاجهـــم بوحـــدتي زرع النخـــاع بجامعـــة عـــين شـــمس والمركـــز الطبـــي العـــالمي، خـــلال الفتـــرة مـــن أغســـطس، 2019 إلـــى أغســـطس 2021. وســـجلت مـــن المرضـــى نتـــائج الفحـــص الســـريري، والنتـــائج المخبريـــة، والاختبـــارات الجينيـــة، والقيـــاس بجهـــاز فلوســـيتوميتر, وتـــم متابعـــة المرضـــي بعـــد الـــزرع للوقـــوف علـــي نســـب حـــدوث ارتـــداد المـــرض، مهاجمـــة الخلايـــا المزروعـــة لجســـد المـــريض،

معدل الأصابة بالبكتريا أو الفيروسات، ومعدل الوفيات والحياة بدون ارتداد المرض.

نتائج البحث: كان هناك فارق كبير ذو دلالة إحصائية بين المجموعات المدروسة فيما يتعلق بنسبة معدل العدوي البكتيرية والفيروسية والذي كان معدل حدوثه أعلي في المجموعة التي أعطيت مضاد الجلوبولين المناعي. وقد وجد فارق كبير كبير ذو دلالة إحصائية بين المجموعات التي خضعت للدراسة فيما يتعلق بداء مهاجمة الخلايا لجسد المريض والتي كان نسبة حدوثها أقل في المجموعة التي أعطيت مضاد الجلوبولين التيموسي. ووجد أيضاً فارق كبير ذو دلالة إحصائية بين المجموعات المدروسة فيما يتعلق بارتداد المرض. وقد وجد فارق كبيرإحصائياً بين المجموعات المدروسة فيما يتعلق بمعدل انزراع أو فشل الخلايا.

الاستنتاج: يقلل مضاد الجلوبولين المناعي من نسب وكذلك حدة حدوث داء مهاجمة الخلايا لجسد المريض، ولكن قد يزيد من معدل الاصابة بالعدوي البكتيرية والفيروسية والفطرية.

الكلمات الدالة: زرع خلايا النخاع الجذعية، مضاد الجلوبولين المناعي، داء مهاجمة الخلايا لجسد المريض.