# CHAPTER 6

## IMMUNOSUPPRESSANT USE AND ALLOIMMUNIZATION AGAINST RED BLOOD CELL TRANSFUSIONS

*Manuscript in preparation*

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*Key words: Alloimmunization, corticosteroids, immunosuppressants, red cell antibodies, transfusions*

## ABSTRACT

### Introduction

Patients receiving red blood cell transfusions are at risk of developing alloantibodies against donor red cell antigens. The risk of alloimmunization is dependent on the number of units administered and patient's genetic predisposition, but has also been suggested to be modulated by a patient's clinical profile. Our aim was to examine whether immunosuppressants suppress the development of clinically relevant RBC antibodies.

### Methods

A two-center retrospective case- referent study was performed where case patients and control patients were sampled from all consecutive patients (17,750 patients) who had received their first and subsequent red cell transfusions in a five year period in the study centers. Cases were all patients with a first detected RBC alloantibody preceded by negative antibody screens. Control patients were two-to-one matched to the case patients on the number of RBC transfusions. Logistic regression analysis was used to examine the association between immunosuppressant exposure and the subsequent occurrence of RBC alloimmunization.

### Results

Among the total study population, 98 patients received immunosuppressive therapy, with 46 patients receiving only corticosteroids, 16 receiving only other immunosuppressants and 36 receiving both. A total of 156 case patients and 312 control patients in the study received a median of 6 transfusions (interquartile ranges 3, 11). The incidence of alloimmunization among patients using immunosuppressants was lower than among other patients receiving red blood cells, adjusted relative rate (RR) 0.46 (confidence interval, CI 0.23-0.89).

### Interpretation

Our findings support a considerably lower risk of alloimmunization with the use of immunosuppressive medications.

## INTRODUCTION

Patients receiving red blood cell transfusions are at risk of developing alloantibodies against donor red blood cell antigens[1]. Alloimmunization against clinically relevant red cell antigens can cause serious complications like acute and delayed hemolytic transfusion reactions. In light of this, it becomes important to study the risk factors associated with alloimmunization in detail, in order to predict which patients are most vulnerable to alloimmunization; and thus they may be considered for more extended matched red blood cell transfusions to prevent alloimmunization. On the other hand identifying clinical factors protecting patients against alloimmunization would be equally important.

The risk of alloimmunization is dependent on the number of red cell units administered[1]. The extent of alloimmunization has been studied in various populations with the incidence of alloimmunization increasing with the number of units, ranging from 7%[1] (after 40 transfused units) to 13%[2] (estimated ) in a general transfused population. The risk of alloimmunization is also determined by a patient's genetic predisposition to form an immune response to these non-self antigens[3]. In addition, it has been suggested that a patient's clinical condition[4] is associated with modulation of the alloimmunization risk. Immunosuppressive therapy could be of particular importance in this respect, because red blood cell transfusions and immunosuppressive therapy often coincide in intensive care, trauma, active autoimmune disorder, cancer and organ transplant patients.

The use of immunosuppressants among a general transfused population and its effect on the risk of clinically relevant RBC alloimmunization, however, has not been reported and was the purpose of this study.



## METHODS

### Design and study population

A matched case-referent study was performed at two study centers- Leiden University Medical Center, Leiden and University Medical Center Utrecht, Utrecht, in the Netherlands. Details of our case- referent study design have been previously described[5]. In short, the source population comprised of all previously non-transfused, non-alloimmunized patients who received their first RBC transfusion at one of the study centers. The study period was January 2005 to December 2010 at Leiden University Medical Center and January 2006 to December 2011 at University Medical Center Utrecht, Utrecht.

Case patients were patients with first time detected clinically relevant red cell antibodies and control patients were patients who did not have clinical red cell antibodies after the same number of transfusions as the matched case. The control sampling was conducted on the principles of a risk-set sampling strategy[6], e.g. for any given case (with *N* units up until alloantibody formation), two control patients with at least the same number of units were randomly selected from the source population (figure 1). Control patients were then matched to case patients on *N* number of units (figure 1). Case and control patients were also matched on the study center[5].

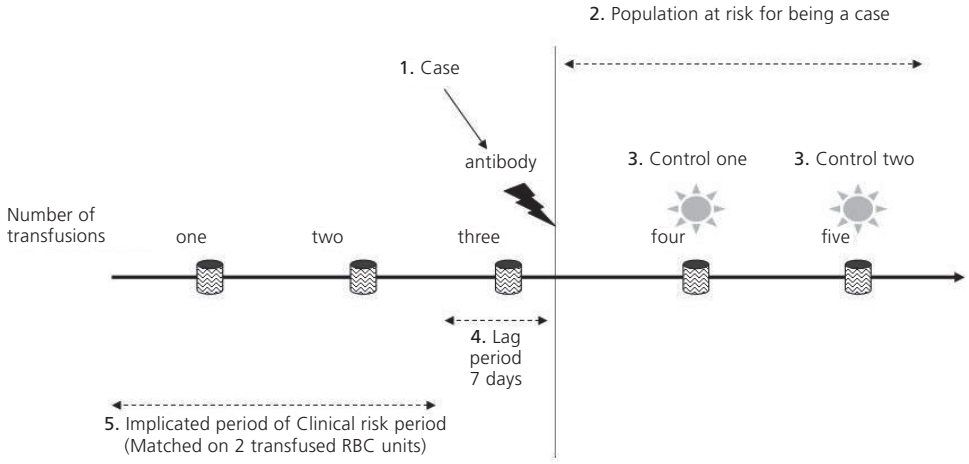


**Figure 1**. Control patient selection and Clinical risk period*

** The chronological order from case patient identification to clinical risk period definition is marked from number 1 to 5.*

The transfusion policy in the study centers was as follows: 1) routinely transfused RBC concentrates were in SAGM and pre-storage leukoreduced and 2) all patients were routinely screened for alloantibodies before transfusion which was repeated at least every 72 hours, if further transfusions were required.

## Clinical risk period (Implicated Period) of alloimmunization

We first set out to define an 'immunization risk' period preceding the antibody finding in order to identify the concurrent clinical conditions that in combination with an antigen mismatched transfused unit (implicated unit) could have led to alloimmunization. We measured all the study variables within this clinical risk period.

For the case patients, this risk period[5,7] was defined as a 30 day period preceding the date of the transfusion immediately before) the first positive alloantibody screen[5]. We chose the risk period not to include the week just before the positive screen to "give" antibodies at least one week to develop. The risk period definition is illustrated in Figure 1. A similar clinical risk period was defined for the control patients, as the period of 30 days preceding the transfusion, at which case and control patients had been matched (figure 1).

Using the above defined method to pick a clinical risk period (the so called implicated period) of alloimmunization, we found in the majority (88%) of our case patients at least one transfusion with the mismatched antigen in the risk period immediately preceding the antibody identification. For the remainder of case patients, we looked further back into their transfusion history to identify the mismatched antigen transfusion unit and re-defined the implicated period as per the above mentioned[5] definition of implicated period, around that particular mismatched transfusion.

There were 20 cases with antibodies like Fya, Jka, M for whom an antigen mismatched red blood cell unit could not be confirmed. This is because the donor red cell units are usually not typed for antigens except for ABO, D, other rhesus (C, E, c, e) and K antigens.

## First time formed clinically relevant red cell alloantibodies

Red cell alloantibodies were defined as warm reacting clinically significant antibodies (C, E, c, e, $C^w$, K, $Fy^a$, $Fy^b$, $Jk^a$, $Jk^b$, $Le^a$, $Le^b$, $Lu^a$, $Lu^b$, M, S and s), and were screened for using a three cell panel including an indirect antiglobulin test (LISS Diamed ID gel system) throughout the study period. Positive screening in the three cell panel led to subsequent identification of the antibody or antibodies by a standard 11 cell panel using the same technique.

Alloantibodies of other specificities than those mentioned, as well as cold reacting alloantibodies are not routinely detected by the three cell panel screening method and thus were not considered to be included as cases of clinical alloimmunization.

## Medication classification

To classify the immunosuppressive therapy into corticosteroids and other immunosuppressants categories (table 1), the World Health Organization's ATC (Anatomical Therapeutic Chemical) classification index was used (source: http://www.whocc.no/atc_ddd_index/). Medications classified under category H, sub-category H02 were included as corticosteroids; medications classified under category L, sub-category L04 were included as (other) immunosuppressants (table 1).



Table 1. Immunosuppressive medication use among total study population of 468 patients

| Immunosuppressive medications (98 patients) | |
|---|---|
| Number of patients (%)<br>Using corticosteroids | |
| Prednisolone | 50 (51) |
| Prednisone | 15 (15) |
| Dexamethasone | 12 (12) |
| Triamcinolone | 5 (5) |
| Hydrocortisone | 4 (4) |
| Methylprednisolone | 1 (1) |
| Betamethasone | 1 (1) |
| **Using other immunosuppressants** | |
| Cyclosporine | 34 (34) |
| Mycofenolaat mofetil | 22 (23) |
| Azathioprine | 3 (3) |
| Lenalidomide | 2 (2) |
| Everolimus | 1 (1) |
| Methotrexat | 1 (1) |
| Thalidomide | 1 (1) |

## Data Collection and Definitions

Transfusion dates, results of the antibody investigations, patients' dates of birth, gender, chronic obstructive pulmonary disease (COPD), infections (bacterial, viral, fungal- diagnosed by laboratory serological techniques including blood and tissue cultures), fever (temperature above 38 degree Celsius), transplants (organ and stem cell), allergies (food, dust, animal and chemical), autoimmune diseases (including rheumatoid arthritis), leukemia (acute lymphoblastic, chronic lymphocytic, acute myeloid, juvenile myelomonocytic, myelodysplastic syndrome and myeloma), lymphoma, chemotherapy (yes or no), surgeries (thoracic, abdominal, cranial and facial, upper and lower limbs and excluding coronary bypass and transluminal angiography), traumas (high impact traumas including cars, motorbikes and bicycles; falls) and diabetes (type 1 and type 2) were collected from clinical files within the defined clinical risk period (implicated period) of alloimmunization. Immunosuppressive medications- corticosteroids and other immunosuppressants used within this risk period were gathered from the hospitals' electronic patient dossiers and information management systems.

## Data Analyses

Specific corticosteroids and other immunosuppressants types and their usage (in numbers and percentages) were presented.

The association between the use of immunosuppressive medications and alloimmunization was modeled using a logistic regression model. Odds ratios were interpreted as relative rates throughout the manuscript. All relative rates (RR) were corrected for the matching factors- total number of transfusions and study center and presented with a 95% confidence interval (CI).

We compared patients receiving 1) any immunosuppressives, 2) exclusively corticosteroids, 3) exclusively other immunosuppressants and 4) exclusively both of these in combination, to patients not exposed to any of these medications, within the implicated period.

The distribution of potential confounders in controls with and without corticosteroids and other immunosuppressants as well as among case patients and total study population (presented in the appendix) were presented in numbers and percentages, or median with interquartile range (IQR).

The adjusted relative rates were adjusted for – sex and age (categorical with <=25, 26-50, 50-75 and > 75 year categories), chronic obstructive pulmonary disease (COPD, infection, fever, transplant, allergies, auto-immune diseases, rheumatoid arthritis (RA), leukemia, lymphoma, chemotherapy, surgery, trauma and diabetes type 1 and type 2 during the implicated period.

# RESULTS

## Characteristics of the study population

Out of a total of 17,750 transfused patients, 468 patients were studied (156 case patients, 312 control patients). 56% (261) patients were from Utrecht and 44% (207) patients were from the Leiden study center. The study population had a median age of 59 years, (IQR 38,

70) and comprised of 56% males. Cases had received a median of 6 units of red cells (IQR 3, 11; range 1-66) units before antibody formation. Antibodies were detected for the first time after a median of 123 days (IQR 25, 333) following the first transfusion.

### Use of immunosuppressive therapy in the clinical risk period (implicated period):

A total of 98 patients used any immunosuppressant medications. Prednisone (50%), prednisolone (15%) and dexamethasone (12%) were the most used corticosteroids and cyclosporine (34%) and mycofenolaat mofetil (22%) the most used other immunosuppressants (table 1).

*Control patients* using immunosuppressive medications (in the month before the matched transfusion) were more often females, 51% to 43% and younger (48 vs. 62 years) as compared to the patients not exposed to immunosuppressive medications. Patients exposed to immunosuppressive medications more often had infections (51% to 24%), fever (33% to 23%), transplants (36% to 3%), allergies (12% to 4%), leukemia (28% to 7%), lymphoma (7% to 4%), chemotherapy (17% to 14%); and a lower percentage of auto-immune diseases (1.3% to 3%), surgeries (37% to 58%) and traumas (none to 9%) compared to patients not using immunosuppressive medications (table 2). The distribution of diabetes type 1 (2% to 1%) and type 2 (8% to 9%) was similar in both patient populations.

Similar group distributions for case patients and the total study population were presented as well (Appendix table 1 and table 2).

### Immunosuppressives and risk of alloimmunization

Eight patients were left out of the adjusted multivariable analysis due to missing data on at least one confounder. Patients receiving immunosuppressive medications had a lower alloimmunization rate than those not receiving these medications. The crude relative rate (RR) was 0.50 (95% CI, 0.29-0.88) and the adjusted RR 0.46 (95% CI 0.23-0.89). With these results, we analyzed specifically patients using only corticosteroids, only other immunosuppressants or both (table 3).

Compared with patients not using any immunosuppressive medications, patients using only corticosteroids, only other immunosuppressants and patients using both all had a lower alloimmunization rate, an adjusted RR 0.52 (95% CI 0.23-1.16); 0.24 (95% CI 0.05-1.20) and 0.52 (95% CI 0.19-1.40) respectively (table 3).

## INTERPRETATION AND DISCUSSION

In our case referent study among previously non transfused, non alloimmunized patients, exposure to immunosuppressives was associated with a lower incidence of clinically relevant red cell alloantibodies against donor red blood cells.

To appreciate these findings, several aspects need to be discussed. Strength of our study is the control sampling strategy. By using a risk-set sampling strategy, our control patients formed a representative sample of the source population. In this study we

**Table 2.** Patient characteristics as observed during the clinical risk period, according to their exposure to immunosuppressive medications; Among *control patients* (n= 312)

| Patient Characteristics***, n (%) | None<br>n= 237 | Corticosteroids or other Immunosuppressant<br>n= 75 | Only Corticosteroids<br>n= 35 | Only other Immunosuppressants<br>n= 14 | Both<br>n= 26 |
|---|---|---|---|---|---|
| Sex, males | 136 (57.4) | 37 (49.4) | 15 (42.9) | 7 (50) | 15 (57.7) |
| Age* (years) | 62 (43, 73) | 48 (29, 59) | 51 (32, 64) | 36 (14, 49) | 51 (29, 63) |
| COPD | 7 (3.0) | 4 (5.5) | 2 (5.9) | 0 (0.0) | 2 (7.7) |
| Infection** | 57 (24.1) | 38 (50.7) | 17 (48.6) | 6 (42.9) | 15 (57.7) |
| Fever | 55 (23.2) | 25 (33.3) | 11 (31.4) | 7 (50.0) | 7 (26.9) |
| Transplants (organ and stem cell) | 7 (3.0) | 27 (36.0) | 9 (25.7) | 6 (42.9) | 12 (46.2) |
| Allergies | 10 (4.2) | 9 (12.0) | 4 (11.4) | 1 (7.1) | 4 (15.4) |
| Auto-immune diseases (including Rheumatoid Arthritis) | 5 (2.1) | 1 (1.3) | 1 (2.9) | 0 (0.0) | 0 (0.0) |
| Leukemia | 17 (7.2) | 21 (28.0) | 11 (31.4) | 5 (35.7) | 5 (19.2) |
| Lymphoma | 9 (3.8) | 5 (6.7) | 2 (5.7) | 2 (14.3) | 1 (3.8) |
| Chemotherapy | 32 (13.5) | 13 (17.3) | 10 (28.6) | 2 (14.3) | 1 (3.8) |
| Surgeries | 138 (58.2) | 28 (37.3) | 14 (40.0) | 3 (21.4) | 11 (42.3) |
| Trauma | 21 (8.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Diabetes Type 1 | 5 (2.1) | 1 (1.3) | 0 (0.0) | 1 (7.1) | 0 (0.0) |
| Diabetes Type 2 | 19 (8.0) | 7 (9.3) | 3 (8.6) | 2 (14.3) | 2 (7.7) |

Values are number (%) or *median (interquartile range)
** includes bacterial, viral and fungal infections
*** all characteristics were measured during the implicated period

Table 3. Relative rate of alloimmunization in patients using only corticosteroids, only other immunosuppressants and both as compared to using none of these

| | Case patients | Control patients | Crude RR* (95% CI) | Adjusted RR** (95% CI) |
|---|---|---|---|---|
| None | 133 | 237 | 1 (ref) | 1 (ref) |
| Only Corticosteroids | 11 | 35 | 0.52 (0.24-1.12) | 0.52 (0.23-1.16) |
| Only Immunosuppressant | 2 | 14 | 0.23 (0.05-1.08) | 0.24 (0.05-1.20) |
| Both | 10 | 26 | 0.64 (0.28-1.43) | 0.52 (0.19-1.40) |

* adjusted for number of matched transfusions and hospital
** adjusted for number of matched transfusions and hospital; sex, age, COPD, infection, fever, transplants, allergies auto-immune diseases, leukemia, lymphoma, chemotherapy, surgeries, trauma diabetes type 1 and diabetes type 2.

RR- Relative Rate; 95% CI- 95% Confidence Interval

examined the combined immune modulating effects of transfusion exposure and that of immunosuppressives administered in the defined implicated period. For this purpose, we carefully chose an implicated period. The aim of defining this clinical risk period in which the transfusion mediated exposure to mismatch antigens occurred, was to enable us to study clinical concurrent events with possible immune modulating effects. While the observed protective association between immunosuppressive therapy and alloimmunization may in part be the result of other risk factors for alloimmunization that are also associated with the use of immunosuppressants i.e. confounding factors, we carefully measured all other risk factors and adjusted for them in our analyses.

Although the possibility of unknown transfusions at a different hospital cannot be entirely ruled out by our strategy, all selected patients needed to have a negative antibody screen preceding the first transfusion and at least followed by one post transfusion antibody screen. This strategy is not totally excluding secondary ("boostered") immune responses. We, however, do not expect this to affect our study findings. There is no reason to believe that patients with unknown previous transfusions and with unknown previous antibodies are more likely to be exposed (or unexposed) to any of the potential confounding variables. The same reasoning is true for the fact that we could not exclude patients with possible previous transfusion history in other hospitals, due to absence of such information in the transfusion records of the study centers.

To our knowledge this is the first study in humans that shows the presence and extent of the protective effect of immune suppressive medications on alloimmunization against clinically relevant red cell antigens. A causal nature of the observed association with use of immunosuppressants is biologically plausible. Their role in suppressing the transplant rejection in the patients undergoing organ transplants[8] has been documented. In addition, immunosuppressive therapy has been shown to impair humoral immune responses to vaccines[9] and antigens[10]. With respect to corticosteroids, hydrocortisone has been shown to diminish in vitro responses to streptokinase- streptodornase and tetanus toxoid[11] vaccinations

as indication of a suppressed immune response. This diminished immune response in presence of corticosteroids has been attributed to transient lymphocytopenia, by the redistribution of circulating T-cells to other body compartments[12]. It has been also demonstrated that proliferation of T-cells can be inhibited by corticosteroids[13-18]. For example, glucocorticoids inhibit production of T-cell growth factor and block the clonal expansion necessary to amplify a primary response[16,19,20].

Other immunosuppressive drugs also suppress T-cell responses[21]. Proliferation of B and T lymphocytes is inhibited by immunosuppressants like mycophenolate[22] and rituximab[10]; while drugs like cyclosporine and tacrolimus inhibit the activation and differentiation of T-cells by inhibiting calcineurin. In addition, a lower influenza vaccine antibody response and diminished T-cell proliferation responses have been shown in with these drugs immunosuppressed liver transplant patients[23].

Considering the mechanisms of the studied alloimmunization against red cell antigens, they are both B- and T helper cell dependent. Although the short lived formation of non-naturally occurring IgM antibodies by B-cell derived plasma cells is mainly T-cell independent, the subsequent memory B-cell response and the formation of more high affinity IgG is T-cell helper dependent. It is therefore likely that in presence of corticosteroids and the other immunosuppressive drugs, the T-cell mediated responses to donor red cell antigens are impaired. Of course, the observed immunosuppression therapy mediated risk reduction of alloimmunization need not be entirely caused by this therapy but a direct attributive effect is strongly plausible.

Therefore when aiming for an eventual alloimmunization risk prediction on the basis of clinical factors, immunosuppressives might be added to such a prediction risk score. This may enable to distinguish high risk patients for alloimmunization that might benefit from cost effective extended donor blood phenotype matching strategies.

In summary, corticosteroids and other immunosuppressant medications appear to have a considerable protective effect on alloimmunization in patients transfused with donor red blood cells. While immune activating conditions are often the reason to start these drugs and coincide with their use, the inhibiting effect that was observed in our studies might be even an underestimation of the true effectiveness of these drugs to block the alloimmunization response.

## REFERENCE LIST


1. Zalpuri S, Zwaginga JJ, le CS, Elshuis J, Schonewille H, van der Bom JG. Red-blood-cell alloimmunization and number of red-blood-cell transfusions. Vox Sang. 2012 Feb;102(2):144-9.
2. Higgins JM, Sloan SR. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. Blood 2008 Sep 15;112(6):2546-53.
3. Noizat-Pirenne F, Tournamille C, Bierling P, Roudot-Thoraval F, Le Pennec PY, Rouger P, Ansart-Pirenne H. Relative immunogenicity of Fya and K antigens in a Caucasian population, based on HLA class II restriction analysis. Transfusion 2006 Aug;46(8):1328-33.
4. Bauer MP, Wiersum-Osselton J, Schipperus M, Vandenbroucke JP, Briet E. Clinical predictors of alloimmunization after red blood cell transfusion. Transfusion 2007 Nov;47(11):2066-71.

5. Zalpuri S, Zwaginga JJ, van der Bom JG. Risk Factors for Alloimmunisation after red blood Cell Transfusions (R-FACT): a case cohort study. BMJ Open. 2012;2(3).
6. Rothman KJ, Mosquin PL. Confounding after risk-set sampling in the beryllium study of Sanderson et al. Ann.Epidemiol. 2011 Oct;21(10):773-9.
7. Zalpuri S, Schonewille H, Middelburg R, van de Watering L, de VK, Zimring J, van der Bom JG, Zwaginga JJ. Effect of storage of red blood cells on alloimmunization. Transfusion 2013 Mar 11.
8. Fishman JA. Infection in Solid-Organ Transplant Recipients. N Engl J Med 2007 Dec 20;357(25):2601-14.
9. Melmed GY, Agarwal N, Frenck RW, Ippoliti AF, Ibanez P, Papadakis KA, Simpson P, Barolet-Garcia C, Ward J, Targan SR, et al. Immunosuppression impairs response to pneumococcal polysaccharide vaccination in patients with inflammatory bowel disease. Am.J.Gastroenterol. 2010 Jan;105(1):148-54.
10. van der Kolk LE, Baars JW, Prins MH, van Oers MH. Rituximab treatment results in impaired secondary humoral immune responsiveness. Blood 2002 Sep 15;100(6):2257-9.
11. Fauci AS, Dale DC. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin.Invest 1974 Jan;53(1):240-6.
12. Fauci AS, Dale DC. Alternate-day prednisone therapy and human lymphocyte subpopulations. J Clin. Invest 1975 Jan;55(1):22-32.
13. Barnes PJ. Glucocorticosteroids: current and future directions. Br.J.Pharmacol. 2011 May;163(1):29-43.
14. Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. Ann.N.Y.Acad.Sci. 2004 Jun;1024:138-46.
15. Lowenberg M, Verhaar AP, van den Brink GR, Hommes DW. Glucocorticoid signaling: a nongenomic mechanism for T-cell immunosuppression. Trends Mol.Med 2007 Apr;13(4):158-63.
16. Liberman AC, Druker J, Refojo D, Holsboer F, Arzt E. Glucocorticoids inhibit GATA-3 phosphorylation and activity in T cells. FASEB J 2009 May;23(5):1558-71.
17. Barshes NR, Goodpastor SE, Goss JA. Pharmacologic immunosuppression. Front Biosci. 2004 Jan 1;9:411-20.
18. Gillis S, Crabtree GR, Smith KA. Glucocorticoid-induced inhibition of T cell growth factor production. II. The effect on the in vitro generation of cytolytic T cells. J Immunol. 1979 Oct;123(4):1632-8.
19. Crabtree GR, Gillis S, Smith KA, Munck A. Mechanisms of glucocorticoid-induced immunosuppression: inhibitory effects on expression of Fc receptors and production of T-cell growth factor. J Steroid Biochem. 1980 Jan;12:445-9.
20. Crabtree GR, Gillis S, Smith KA, Munck A. Glucocorticoids and immune responses. Arthritis Rheum. 1979 Nov;22(11):1246-56.
21. Larsson EL. Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at distinct sites of the triggering process. J Immunol. 1980 Jun;124(6):2828-33.
22. Ransom JT. Mechanism of action of mycophenolate mofetil. Ther.Drug Monit. 1995 Dec;17(6):681-4.
23. Soesman NM, Rimmelzwaan GF, Nieuwkoop NJ, Beyer WE, Tilanus HW, Kemmeren MH, Metselaar HJ, de Man RA, Osterhaus AD. Efficacy of influenza vaccination in adult liver transplant recipients. J Med Virol. 2000 May;61(1):85-93.

# APPENDIX

**Appendix table** 1. Patient characteristics according to use of immunosuppressive medications; Among *total study population* (n= 468)

| Patient Characteristics***, n (%) | None n=370 | Corticosteroids or other Immunosuppressant n= 98 | Only Corticosteroids n= 46 | Only other Immunosuppressants n= 16 | Both n= 36 |
|---|---|---|---|---|---|
| Sex, males | 211 (57) | 50 (51) | 20 (44) | 9 (56) | 21 (58) |
| Age* (years) | 62 (43-73) | 49 (29-60) | 51 (34, 63) | 28 (9, 48) | 52 (23, 63) |
| COPD | 14 (3.8) | 4 (4.2) | 2 (4.4) | 0 (0) | 2 (5.7) |
| Infection** | 95 (25.7) | 51 (52) | 21 (45.7) | 8 (50) | 22 (61.1) |
| Fever | 84 (22.7) | 34 (34.7) | 13 (28.3) | 8 (50) | 13 (36.1) |
| Transplants (organ and stem cell) | 10 (2.7) | 36 (36.7) | 10 (21.7) | 6 (37.5) | 20 (55.6) |
| Allergies | 18 (4.9) | 12 (12.2) | 5 (10.9) | 1 (6.2) | 6 (16.7) |
| Auto-immune diseases (including Rheumatoid Arthritis) | 7 (1.9) | 2 (2.0) | 1 (2.2) | 0 (0.0) | 1 (2.8) |
| Leukemia | 26 (7) | 26 (26.5) | 13 (28.3) | 6 (37.5) | 7 (19.4) |
| Lymphoma | 10 (2.7) | 5 (5.1) | 2 (4.3) | 2 (12.5) | 1 (2.8) |
| Chemotherapy | 43 (11.6) | 19 (19.4) | 15 (32.6) | 2 (12.5) | 2 (5.6) |
| Surgeries | 223 (60) | 37 (37.8) | 19 (41) | 3 (19) | 15 (42) |
| Trauma | 33 (8.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Diabetes Type 1 | 5 (1.4) | 1 (1.0) | 0 (0.0) | 1 (6.2) | 0 (0.0) |
| Diabetes Type 2 | 38 (10.3) | 10 (10.2) | 4 (8.7) | 2 (12.5) | 4 (11.1) |

Values are number (%) or *median (interquartile range)
** includes bacterial, viral and fungal infections
*** all characteristics were measured during the implicated period

**Appendix table** 2. Patient characteristics according to use of immunosuppressive medications; Among all *case patients* (n= 156)

| Patient Characteristics***, n (%) | None<br>n= 133 | Corticosteroids or other Immunosuppressant<br>n= 23 | Only Corticosteroids<br>n= 11 | Only other Immunosuppressants<br>n= 2 | Both<br>n= 10 |
|---|---|---|---|---|---|
| Sex, males | 75 (56.4) | 13 (56.5) | 5 (45.5) | 2 (100.0) | 6 (60.0) |
| Age* (years) | 61 (39-72) | 51 (26-62) | 51 (39-62) | 10 (7-10) | 53 (21-64) |
| COPD | 7 (5.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Infection** | 38 (28.6) | 13 (56.5) | 4 (36.4) | 2 (100.0) | 7 (70.0) |
| Fever | 29 (21.8) | 9 (39.1) | 2 (18.2) | 1 (50.0) | 6 (60.0) |
| Transplants (organ and stem cell) | 3 (2.3) | 9 (39.1) | 1 (9.1) | 0 (0.0) | 8 (80.0) |
| Allergies | 8 (6.0) | 3 (13.0) | 1 (9.1) | 0 (0.0) | 2 (20.0) |
| Auto-immune diseases (including Rheumatoid Arthritis) | 2 (1.5) | 1 (4.3) | 0 (0.0) | 0 (0.0) | 1 (10.0) |
| Leukemia | 9 (6.8) | 5 (21.7) | 2 (18.2) | 1 (50.0) | 2 (20.0) |
| Lymphoma | 1 (0.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Chemotherapy | 11 (8.3) | 6 (26.1) | 5 (45.5) | 0 (0.0) | 1 (10.0) |
| Surgeries | 85 (63.9) | 9 (39.1) | 5 (45.5) | 0 (0.0) | 4 (40.0) |
| Trauma | 12 (9.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Diabetes Type 1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Diabetes Type 2 | 19 (14.3) | 3 (13.0) | 1 (9.1) | 0 (0.0) | 2 (20.0) |

Values are number (%) or *median (interquartile range)
** includes bacterial, viral and fungal infections
*** all characteristics were measured during the implicated period