Staphylococcus Aureus carriage and long-term Rituximab treatment for Granulomatosis with polyangiitis

Emilio Besada, Wenche Koldingsnes, Johannes C Nossent

Objective: Chronic nasal carriage of Staphylococcus aureus (SA) increases the risk of relapse while Rituximab (RTX) is an effective agent for inducing and maintaining remission in patients with Granulomatosis with polyangiitis (GPA). We investigated whether B cell depletion and hypogammaglobulinemia that occur during RTX treatment increase the risk of chronic SA nasal carriage and subsequent disease flares, in GPA patients on long-term RTX maintenance therapy. Methods: Retrospective cohort study from a disease registry involving 29 GPA patients receiving RTX maintenance (median RTX dose of 9 g) during a median period of 49 months. Nasal swabs were collected prior and during RTX for a median of 3 and 9 swabs respectively. Persistent SA nasal carriage was defined with the presence of SA in more than 75 % of nasal swabs. Results: SA nasal carriage did not change during RTX (p=0.297). However, the rate of positive nasal swabs in GPA patients with transient SA nasal carriage during RTX maintenance increased from 0 prior RTX to 0.42 during RTX (p=0.017). Persistent SA nasal carriage did not increase the risk of relapses (p=0.844), of hypogammaglobulinemia (p=0.122) and of severe infections (p=0.144), but reduced the risk of chronic infections (p=0.044). Change in SA carriage status during RTX did not influence the risk of relapses (p=0.756), hypogammaglobulinamia (p=0.474) and infections, either severe (p=0.913) or chronic (p=0.121). Conclusion: Long-term RTX maintenance therapy in GPA patients did not significantly influence SA nasal carriage status. Persistent SA carriage during long-term RTX treatment did not seem to increase the risk of relapses, but seemed to decrease the risk of hypogammaglobulinemia associated chronic infections.

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23

26   ABSTRACT

28   Objective:

29   Chronic nasal carriage of Staphylococcus aureus (SA) increases the risk of relapse while

30   Rituximab (RTX) is an effective agent for inducing and maintaining remission in patients with

31   Granulomatosis with polyangiitis (GPA). We investigated whether B cell depletion and

32   hypogammaglobulinemia that occur during RTX treatment increase the risk of chronic SA nasal

33   carriage and subsequent disease flares, in GPA patients on long-term RTX maintenance therapy.

34   Methods:

35   Retrospective cohort study from a disease registry involving 29 GPA patients receiving RTX

36   maintenance (median RTX dose of 9 g) during a median period of 49 months. Nasal swabs were

37   collected prior and during RTX for a median of 3 and 9 swabs respectively. Persistent SA nasal

38   carriage was defined with the presence of SA in more than 75 % of nasal swabs.

39   Results:

40   SA nasal carriage did not change during RTX (p=0.297). However, the rate of positive nasal

41   swabs in GPA patients with transient SA nasal carriage during RTX maintenance increased from

42   0 prior RTX to 0.42 during RTX (p=0.017).

43   Persistent SA nasal carriage did not increase the risk of relapses (p=0.844), of

44   hypogammaglobulinemia (p=0.122) and of severe infections (p=0.144), but reduced the risk of

45   chronic infections (p=0.044). Change in SA carriage status during RTX did not influence the risk

46   of relapses (p=0.756), hypogammaglobulinamia (p=0.474) and infections, either severe

47   (p=0.913) or chronic (p=0.121).

48   Conclusion:

49   Long-term RTX maintenance therapy in GPA patients did not significantly influence SA nasal

50   carriage status. Persistent SA carriage during long-term RTX treatment did not seem to increase

51   the risk of relapses, but seemed to decrease the risk of hypogammaglobulinemia associated

52   chronic infections.

54   INTRODUCTION

55   Granulomatosis with polyangiitis (GPA) is a clinicopathologic variant of antineutrophil

56   cytoplasmic antibodies (ANCA)-associated vasculitis (AAV). It is characterized by a necrotizing

57   granulomatous inflammation usually involving the upper and lower respiratory tract, a

58   necrotizing vasculitis affecting predominantly small to medium vessels and commonly a

59   necrotizing glomerulonephritis, often in the presence of Proteinase 3 (PR3)-ANCA [1]. The

60   aetiology of GPA is unknown; but infections and in particular nasal carriage of Staphylococcus

61   aureus (SA) have been implicated [2]. SA nasal carriage may trigger GPA through

62   staphylococcal superantigens, molecular mimicry, increased Toll-like receptor signalling in

63   leukocytes and the induction of neutrophil extracellular traps (NETs) [2]. SA interacts with

64   endothelial, T and B cells creating an inflammatory milieu and activating neutrophils locally

65   responsible of damage by release of proteolytic enzymes and reactive oxygen species [3]. SA can

66   evade innate immunity by resisting phagocytosis and can survive inside neutrophils [4]. SA can

67   also evade induced immunity since SA can deplete potential antibody-secreting B cells and

68   inhibit proliferation of antigen-specific T cells [4]. GPA patients have an abnormal epithelial

69   nasal barrier with an altered basal cytokine expression and a reduced secretion of interleukin-8

70   upon SA stimulation that could facilitate nasal carriage [5].

71   SA permanently colonises the anterior nares of 20 % of the population while transient

72   colonisation occurs in another 60 % [6]. In one study, GPA patients have a slightly increased rate

73   of SA nasal carriage (72 %), compared to Rheumatoid Arthritis patients (46 %) and hospital staff

74   members (58 %) [7]. The risk of disease relapses in GPA increases with SA nasal carriage [7]

75   and is 7 times higher with chronic SA nasal carriage [8]. Antibiotic prophylaxis with

76   Trimethoprim-sulfamethoxazole (TMP-SMX) can decrease the risk of relapse by 60 % and of

77   infections in GPA [9,10], although the basis for this is unclear since TMP-SMX antibiotic

78   prophylaxis does not eliminate SA nasal carriage in GPA [5,11].

79   While immunosuppressive drugs (ID) are the mainstay of therapy in GPA, B cell depleting

80   therapy with rituximab (RTX) has proven very effective in inducing [12,13] and maintaining

81   remission in GPA [14-16]. However, side effects of B cell depletion over time such as the

82   reduction in all immunoglobulins (Ig) class levels [16] could potentially increase the risk of SA

83   carriage.

84   Our study investigated if long-term RTX maintenance treatment increases the risk of SA carriage

85   in GPA patients and if SA carriage promoted relapses, infections and/or

86   hypogammaglobulinemia.

88   PATIENTS AND METHODS

89   Since 2001 our vasculitis disease registry (Nordnorsk Vaskulittregister) has collected

90   information on disease presentation and course from patients with an established diagnosis of

91   primary vasculitis followed at the University Hospital of North Norway. All patients gave

92   informed written consent at registry inclusion according to the declaration of Helsinki.

93   All patients satisfied the American College of Rheumatology 1990 classification and/or Chapel

94   Hill consensus conference criteria [17-18]. Twenty-nine GPA patients from that registry who

95   received long-term RTX treatment with a RTX cumulative dose of 5 g or more before September

96   2011 were included in the study. 86 % received RTX for relapse and 14 % for new disease.

97   RTX treatment was initiated as two 1-gram infusions 2 weeks apart with co-administration of

98   methylprednisolone 125 mg, paracetamol 1000 mg and either cetirizine 10 mg or polaramine 4

99   mg (RA protocol). Due to the observed RTX efficacy and the relapsing nature of GPA, RTX was

100   then re-administered pre-emptively either as 2 g infusion (1 g twice during a fortnight) annually

101   or as 1 g infusion biannually (1 g every 6 months). RTX was added to other ID (other than

102   prednisolone) in 27 patients (93 %), while 2 (7%) received RTX in monotherapy. The timing and

103   pace of ID discontinuation was at the discretion of the treating physician, whereas the oral daily

104   prednisolone dose (ODPD) was tapered and discontinued in a controlled manner.

105

106   Antibiotic prophylaxis against relapse with TMP-SMX (dose 320mg of TMP and 1600mg of

107   SMX daily) was not blinded and was at the physician's discretion. Ciprofloxacin (dose ranging

108   from 500 to 1000 mg daily) was used in case of sulfamide allergy, poor TMP-SMX tolerance or

109   concomitant methotrexate use. Compliance of antibiotic prophylaxis was assessed during

110   patients' visits. We defined antibiotic prophylaxis as the use of antibiotic prophylaxis for more

111   than 50 % of the time during long-term RTX maintenance.

112   Nasal swabs were performed biannually in one anterior naris by firmly rotating the sterile cotton-

113   swab. Similar to the study of Stegeman el al, we defined patients with positive SA nasal carriage

114   as either transient or persistent if SA was present in respectively less than 75 % and more than 75

115   % of all the nasal swabs [8]. We defined patients as non-SA carriers when none of the nasal

116   swabs grew SA.

117   Fixed set of blood tests (ANCA levels, quantification of immunoglobulin classes and flow

118   cytometric immunophenotyping of lymphocytes) were performed prior RTX initiation and

119   before each new re-treatment until either 30th September 2011 (closing date of this study) or

120   shortly before the administration of intravenous immunoglobulins (IVIG) due to

121   hypogammaglobulinemia and/or infections. CD4 cell counts and serum total Ig levels taken after

122   the administration of 2 g of RTX and at last visit were included in the study.

123

124   Relapses were defined by the recurrence, worsening or first appearance of one or more BVAS

125   items attributable to GPA after remission. Hypogammaglobulinemia was defined as serum total

126   Ig < 6 g/L. Severe infections were defined as if hospitalisation and intravenous antibiotic

127   treatment were necessary, while chronic infections were defined as symptomatic infections

128   mostly localised in the ear-nose-throat (ENT) and respiratory tract, lasting 3 months or more and

129   requiring several antibiotic courses.

130

131   Data were analysed with SPSS version 20.0 (SPSS Ltd, Chicago IL, USA). Descriptive statistics

132   were used to describe SA nasal carriage during RTX maintenance. Continuous variables were

133   expressed in median and range; categorical variables were expressed in number and percentage.

134   Related-samples Wilcoxon signed rank test was used to test for difference between rates of

135   positive SA nasal swabs prior RTX and during maintenance. Chi-square and Kruskal Wallis tests

136   were used appropriately for subgroups analysis concerning SA nasal carriage status. SA nasal

137   carriage, change in SA carriage status and antibiotic prophylaxis during RTX maintenance were

138   analysed by Kaplan-Meier survival curves as risk factors of relapse, infections and RTX

139   discontinuation due to hypogammaglobulinemia. P-values < 0.05 were considered significant.

141   RESULTS

142   Patients' characteristics

143   Twenty-nine GPA patients (52 % men) with a median age of 50 years (19-75) received RTX. 90

144   % of the patients were ANCA positive at diagnosis: 86 % PR3-ANCA and 3 % MPO-ANCA

145   positive. 59 % had renal involvement; 66 % had pulmonary involvement and 62 % had orbital-

146   subglottic involvement. At RTX initiation, their disease duration was 57 (22-70) months and

147   they had received a cumulative dose of 17 (0-250) g cyclophosphamide.

148   GPA patients received a cumulative RTX dose of 9 g (5-13) for remission induction and

149   maintenance during 49 (19-88) months. Twelve patients (41 %) received the 1 g biannually

150   regimen, 6 (21 %) received the 2 g annually regimen and 11 (38 %) alternated between regimens.

151   RTX was added to other ID (other than prednisolone) in 27 patients (93 %), which was

152   continued during 24 months (1-54) after RTX initiation. ODPD decreased from 23 (0-60) at

153   baseline to 5 (0-15) mg at last visit. Six patients (21 %) discontinued prednisolone 23 months (7-

154   55) after RTX initiation.

155

156

157   Nasal carriage during RTX maintenance

158   Three (1-8) nasal swabs were analysed before RTX and 9 (2-22) during RTX maintenance. The

159   number of nasal swabs did not differ between the different SA carriage groups before and during

160   RTX (respectively p=0.411 and p=0.437).

161   Before RTX initiation, 13 patients (52 %) were non-SA carriers, but 2 patients had positive

162   swabs for other bacteria (Table 1). Seven patients (28 %) had transient SA nasal carriage and 5

163   (20 %) had persistent carriage before RTX. During RTX maintenance, 9 patients (31 %) were

164   non-SA carriers, while 12 (41 %) had transient carriage and 8 (28 %) had persistent nasal

165   carriage. Ten (40 %) patients did not change SA carriage status during RTX maintenance, while

166   11 (44 %) increased nasal SA carriage status and 4 (16 %) decreased during RTX maintenance.

167   The frequency of SA nasal carriage did not differ significantly before and during RTX (p=0.297)

168   (Table 1). However, the rate of positive nasal swabs in GPA patients with transient SA nasal

169   carriage during RTX maintenance (n=11) increased from 0 prior RTX to 0.42 during RTX

170   (p=0.017 after square root transformation).

171   The nasal swabs identified several other bacteria at initiation and during RTX maintenance

172   (Table 1). Of interest, 7 (24 %) and 4 (14 %) GPA patients had respectively Streptococcus

173   pneumoniae and Haemophilus influenzae at least once in their nasal swabs during RTX

174   maintenance. S. pneumoniae and SA were often identified together at last visit, whereas H.

175   influenzae was identified in 2 out of 8 patients who discontinued RTX due to

176   hypogammaglobulinemia at the time of its discontinuation.

177

178

179   SA nasal carriage

180   GPA patient characteristics differed in relation to SA nasal carriage. SA non-carriers had less

181   lung involvement (p=0.047) and seemed to receive higher ODPD at baseline (p=0.141)

182   compared with patients with either transient or persistent nasal carriage (Table 2). On the other

183   hand, patients with persistent SA nasal carriage had received 55 g of CYC compared with 13 g in

184   the other 2 subgroups (p=0.163). The use of other ID during RTX treatment was similar within

185   the different SA nasal carriage subgroups (Table 2).

186

187   Risk of relapse

188   During RTX maintenance, 9 relapses occurred in 8 patients (28 %) and 2 thirds of the relapses

189   involved the respiratory tract and ENT. Three patients (38 %) who relapsed had persistent SA

190   nasal carriage (Table 2). The lone patient who relapsed twice did not carry SA. There was no

191   association between SA nasal carriage and either overall frequency of relapses (p=0.243) (Table

192   2) or time-adjusted risk of relapse (p=0.844) (Figure 1A). There was no association between

193   change of SA nasal carriage status during RTX and time-adjusted risk of relapse (p=0.756)

194   (Figure 2A).

195

196   Risk of infections

197   During RTX maintenance, severe and chronic infections developed in 7 and 9 patients

198   respectively.

199   Seven patients developed severe infections, but there was no association between SA carriage

200   and the frequency (p=0.213) (Table 2) or time-adjusted risk (p=0.144) (Figure 1B) of severe

201   infections. There was no association between change of SA nasal carriage status during RTX and

202   time-adjusted risk of relapse (p=0.913) (Figure 2B).

203   Nine patients had chronic infections; however none of these patients had persistent SA nasal

204   carriage (p=0.082) (Table 2). Patients with persistent SA nasal carriage and patients with an

205   increase of SA nasal carriage status seemed to have a lower time-adjusted risk of developing

206   chronic infections (respectively p=0.044 and p=0.125) (Figures 1C and 2C). Of interest, CD4

207   cell count seemed to increase in persistent and transient carriers, but seemed to decrease in non-

208   carriers following the first 2 g of RTX (p=0.071) (Table 2).

209

210   Risk discontinuing RTX due to hypogammaglobulinemia

211   Eight patients discontinued RTX due to hypogammaglobulinemia with a level of serum total Ig

212   of 4.7 (3.5-5.5) g/L. Four patients (50 %) had persistent or transient SA nasal carriage, while 4

213   patients (50 %) did not carry SA (Table 2). Patients with transient SA nasal carriage had a

214   tendency to lower time-adjusted risk of discontinuing RTX due to hypogammaglobulinemia,

215   although this was not statistically significant (p=0.122) (Figure 1D). Change in SA nasal carriage

216   status during RTX did not influence the risk of discontinuing RTX due to

217   hypogammaglobulinemia (p=0.474) (Figure 2D).

218   Non-SA carriers had a tendency to more profound decline of serum total Ig both after the first 2

219   g of RTX (p=0.079) and during RTX maintenance (p=0.063) compared with patients with either

220   transient or persistent SA carriage (Table 2).

221

222

223   TMP-SMX and ciprofloxacin during RTX

224   Before RTX initiation, most patients (90 %) had received antibiotic prophylaxis with either

225   TMP-SMX or ciprofloxacin at some point during the course of their disease. However, only 16

226   patients (55 %) received TMP-SMX antibiotic prophylaxis during RTX maintenance and just 6

227   (21 %) used TMP-SMX for more than 50 % of the time. Similarly, 10 patients (35 %) received

228   ciprofloxacin during RTX maintenance and 7 (24 %) used it for more than 50 % of the time.

229   Two patients (7 %) switched from ciprofloxacin to TMP-SMX, while no patients switched from

230   TMP-SMX to ciprofloxacin during RTX maintenance. The first patient switched when

231   methotrexate was discontinued. The other switched with the intention of preventing relapse when

232   mycophenolate mofetil was discontinued (even though the nasal swab was negative). ODPD,

233   CYC cumulative dose and concomitant use of ID during RTX maintenance did not differ

234   between patients receiving either TMP-SMX or ciprofloxacin and patients not receiving

235   antibiotics.

236

237   During RTX treatment, none of the six GPA patients who used TMP-SMX for more than 50 %

238   of the time had persistent SA nasal carriage and three (50 %) were non-SA carriers. In the

239   ciprofloxacin group, 2 (29 %) had persistent SA nasal carriage and 2 (29 %) were non-SA

240   carriers. Patients who used TMP-SMX for more than 50 % of the time during RTX maintenance

241   had an increased risk to hypogammaglobulinemia leading to RTX discontinuation (p=0.046)

242   (Figure 3A). On the other hand, none of the patients using ciprofloxacin for more than 50 % of

243   the time during RTX maintenance seemed to discontinue RTX due to hypogammaglobulinemia

244   (p=0.065) (Figure 3B). There were no significant differences for the risk of severe infections,

245   chronic infections and relapses risks in patients on or off antibiotic prophylaxis (with either

246   TMP-SMX or ciprofloxacin) (data not shown).

248 DISCUSSION

249 The rate of persistent SA nasal carriage did not change significantly in GPA patients during

250 long-term RTX treatment, even though SA nasal carriage seemed to increase in patients with

251 transient carriage. Persistent SA nasal carriage was not a risk factor for disease relapses, but was

252 associated with a lower frequency of chronic infections. On the other hand, non-SA carriers

253 seemed to have lower CD4 counts and a steeper decline of total Ig after 2g of RTX and were

254 more prone to infections and to discontinue RTX due to hypogammaglobulinemia.

255

256 Contrary to the seminal study of Stegeman et al [8], persistent SA nasal carriage did not increase

257 the risk of relapse in GPA patients whilst on treatment with RTX. This may be due to the lower

258 overall frequency of SA carriers in this cohort since 63 % were persistent SA nasal carriers in the

259 Stegeman study [8], but only 20 % and 28 % persistently carried SA in our cohort before and

260 during RTX maintenance respectively. Nasal swabs procedure and inclusion as well as patient

261 selection bias could explain the difference. Nasal swabs were performed unilaterally in our study

262 and bilaterally in the study of Stegeman [8]. Nasal swabs of GPA patients receiving prolonged

263 antibiotics defined as more than 6 weeks were excluded in the study of Stegeman [8], whereas all

264 nasal swabs in our study were included with or without concomitant antibiotics. Also while

265 Stegeman et al enrolled all consecutive patients diagnosed with GPA over a 3.5 years period [8],

266 our study included consecutive GPA patients receiving long-term RTX maintenance, who had

267 been mostly refractory to conventional therapy.

268

269 The frequency of severe infections was equal in persistent and non-SA carriers, but the risk of

270 chronic infections during RTX treatment seemed lower in persistent SA carriers and in patients

271   with increased SA nasal carriage status. In healthy individuals, persistent SA nasal carriers have

272   a higher risk of infection [19] than transient and non-carriers who eradicate SA faster due to

273   stronger anti-staphylococcal antibodies response [20]. In our study, non-SA carriers

274   paradoxically seemed to have a more profound decline of total serum Ig and CD4 cell count

275   already after 2 g of RTX, indicating an early effect of RTX on the ability to mount an effective

276   immune response in non-SA carriers.

277   In healthy individuals, SA carriage also influences the nasal microbiome and inhibits

278   colonisation from other bacteria [19]. Consequently, persistent SA nasal carriage in GPA patients

279   receiving long-term RTX could prevent colonisation from other bacteria and chronic infections

280   due to the continuing interplay between SA and the immune system.

281

282   In our selected cohort of closely followed GPA patients, antibiotic prophylaxis was neither

283   universal nor continuous and this reflects the uncertainties regarding this issue [7]. TMP-SMX

284   use during RTX maintenance seemed to increase the risk of hypogammaglobulinemia and RTX

285   discontinuation and to decrease persistent SA nasal carriage, compared with ciprofloxacin. While

286   TMP-SMX therapy eliminates neither SA nasal carriage nor endonasal activity in GPA [7], the

287   combination of TMP-SMX and RTX could provoke changes in the nasal epithelial barrier and its

288   microbiome that might be relevant for the course of the disease. Moreover, TMP-SMX could

289   exert an additional anti-inflammatory effect by interfering with the production of reactive oxygen

290   species in neutrophils [21]. As TMP-SMX decreases the risk of relapse [9], it could, in addition,

291   increase the risk to discontinue RTX due to hypogammaglobulinemia.

292

293   Our retrospective study is limited by its sample size with the low number of events making

294   subgroups analysis prone to type II errors of not detecting significant differences. Patients'

295   selection with multiple relapses and confounding factors, such as high CYC cumulative dose,

296   could have biased our results and limit their applicability. Despite this, our study is the first that

297   highlights a possible association between SA carriage status, infections and

298   hypogammaglobulinemia during RTX therapy in GPA patients.

299

300   In conclusion, SA nasal carriage in GPA patients did not change significantly during long-term

301   RTX maintenance. Non-SA carriers were as likely to have disease flares but were more prone to

302   infections and to discontinue RTX due to hypogammaglobulinemia independently of the CYC

303   cumulative dose. These results suggest that long-term RTX treatment is better tolerated in GPA

304   patients with SA nasal carriage.

305

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Staphylococcus aureus and other bacteria nasal carriage before and during rituximab maintenance in granulomatosis with polyangiitis patients

RTX: rituximab Results are expressed in absolute numbers (percentage) and significance is determined by Chi-squared test for association. Footnotes: a. No nasal swabs results were recorded in 4 patients. b. One patient had Pseudomonas aeruginosa prior to RTX. c. One patient had Pseudomonas aeruginosa in 2 nasal swabs and one patient had Neisseria species found in one nasal swab. [b]

2 Table 1

4 Staphylococcus aureus and other bacteria nasal carriage before and during rituximab maintenance in

5 granulomatosis with polyangiitis patients

	Prior RTX (25) [a]	During RTX (29)	p
Staphylococcus aureus			0.297
Persistent carriage	5 (20)	8 (28)	
Transient carriage	7 (28)	12 (41)	
Non carrier	13 (52)	9 (31)	
Haemophilus influenza	0	4 (14)	NA
Streptococcus pneumonia	1 (4)	7 (24)	NA
Other bacteria	1 (4)[b]	2 (7)[c]	NA

8 RTX: rituximab
9
10 Results are expressed in absolute numbers (percentage) and significance is determined by Chi-squared
11 test for association.
12
13 Footnotes:
14 a. No nasal swabs results were recorded in 4 patients.
15 b. One patient had Pseudomonas aeruginosa prior to RTX.
16 c. One patient had Pseudomonas aeruginosa in 2 nasal swabs and one patient had Neisseria species
17 found in one nasal swab.

Granulomatosis with polyangiitis patients' characteristics determined by Staphylococcus aureus nasal carriage during long-term rituximab maintenance.

AZA: azathioprine; BVAS: Birmingham vasculitis activity score; CD: cluster of differentiation; CYC: cyclophosphamide; Ig: immunoglobulins; MMF: mycophenolate mofetil; MTX: methotrexate; PR3-ANCA: proteinase 3 antineutrophil cytoplasmic antibodies; RTX: rituximab; SA: Staphylococcus aureus. TMP-SMX: trimethoprim-sulfamethoxazole Results are expressed in medians for continuous variables and in absolute numbers (percentages) for categorical variables. Difference is determined by Kruskal Wallis Test for continuous variables and Chi-square test for categorical variables.

2   Table 2

3   Granulomatosis with polyangiitis patients' characteristics determined by Staphylococcus aureus nasal

4   carriage during long-term rituximab maintenance.

	No carriage	Transient carriage	Persistent carriage	p-value
	9 patients	12 patients	8 patients	
Male	5 (56)	5 (42)	5 (62)	0.634
Age at baseline (y)	54	43	44	0.375
Kidney involvement	5(56)	6(50)	6(75)	0.525
Lung involvement	**3(33)**	**10(83)**	**6(75)**	**0.047**
Orbital-subglottic involvement	4(44)	9(75)	5(63)	0.361
PR3-ANCA	8(89)	10(83)	7(88)	0.414
BVAS at baseline	11	10	10	0.382
RTX maintenance duration (w)	173	182	170	0.305
CYC cumulative dose (g)	13	13	55	0.163
RTX cumulative dose (g)	8	9.5	8	0.468
1g biannually regimen	6(67)	3(25)	3(38)	0.153
MTX use during RTX	3(33)	4(33)	4(50)	0.711
AZA use during RTX	3(33)	3(25)	2(25)	0.898
MMF use during RTX	1(11)	5(42)	2(25)	0.295
CYC use during RTX	3(33)	3(25)	3(38)	0.891
Total Ig at baseline (g/L)	11	8.5	11	0.278
Total Ig after RTX 2g (g/L)	8.1	7.5	9.4	0.730
CD4 at baseline (x10^9/L)	0.39	0.40	0.27	0.538
CD4 after RTX 2g (x10^9/L)	0.28	0.45	0.39	0.071
Total Ig decline after RTX 2g (g/L)	3.5	1.4	1.9	0.079
Total Ig overall decline during RTX	5.1	2.5	3.4	0.063
TMP-SMX during RTX	3(33)	3(25)	0	0.212
Ciprofloxacin during RTX	2(22)	3(25)	2(25)	0.987
Severe infections	3(33)	1(8)	3(38)	0.243
Chronic infections	4(44)	5(42)	0	0.082
Relapses	2(22)	3(25)	3(38)	0.754
RTX discontinuation due to hypogammaglobulinemia	4(44)	2(17)	2(25)	0.364

7   AZA: azathioprine; BVAS: Birmingham vasculitis activity score; CD: cluster of differentiation; CYC:

8   cyclophosphamide; Ig: immunoglobulins; MMF: mycophenolate mofetil; MTX: methotrexate; PR3-

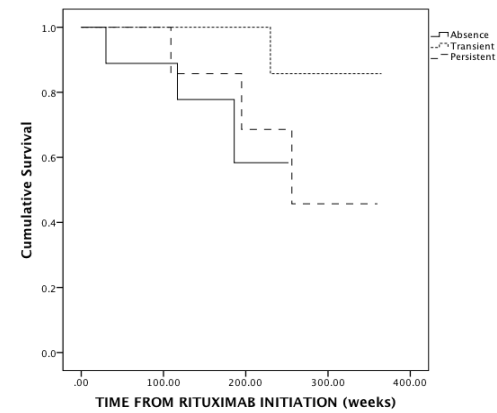
9   ANCA: proteinase 3 antineutrophil cytoplasmic antibodies; RTX: rituximab; SA: Staphylococcus
10 aureus. TMP-SMX: trimethoprim-sulfamethoxazole
11
12 Results are expressed in medians for continuous variables and in absolute numbers (percentages) for
13 categorical variables. Difference is determined by Kruskal Wallis Test for continuous variables and
14 Chi-square test for categorical variables.

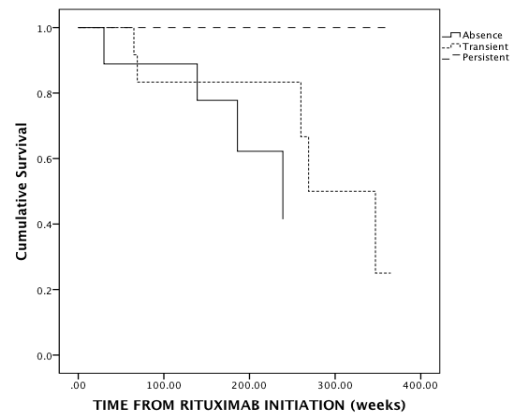
Kaplan-Meier analysis of the probability of relapse (A), severe (B) and chronic (C) infections and discontinuation due to hypogammaglobulinemia (D) according to Staphylococcus aureus nasal carriage status

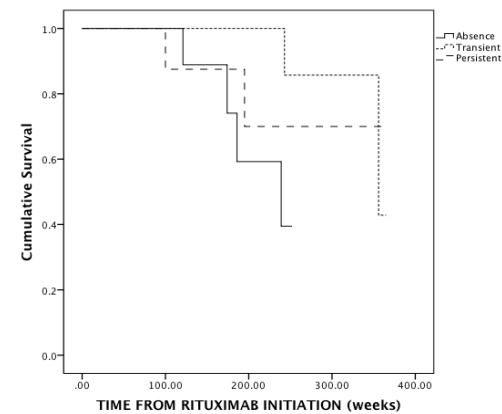
Figure 1

A Log rank p=0.844

Week	0	100	200	300
N	29	22	10	2

B Log rank p=0.144

Week	0	100	200	300
N	29	27	15	4

C Log rank p=0.044

Week	0	100	200	300
N	29	25	15	4

D Log rank p=0.122

Week	0	100	200	300
N	29	28	16	4

Kaplan-Meier analysis of the probability of relapse (A), severe (B) and chronic (C) infections and discontinuation due to hypogammaglobulinemia (D) according to change in Staphylococcus aureus nasal carriage status

Figure 2

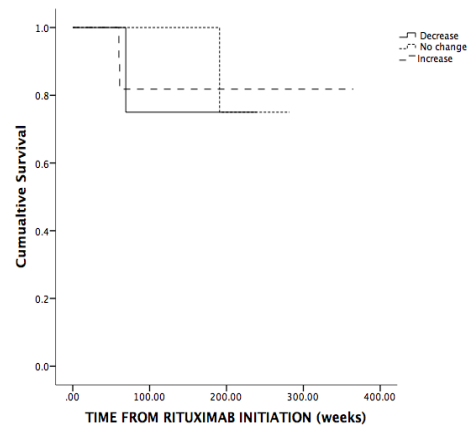
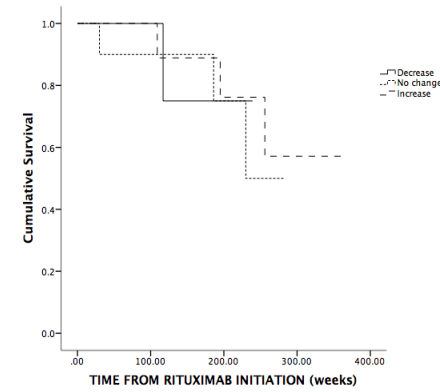
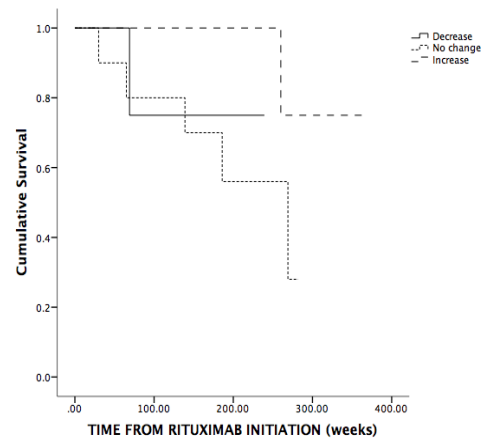
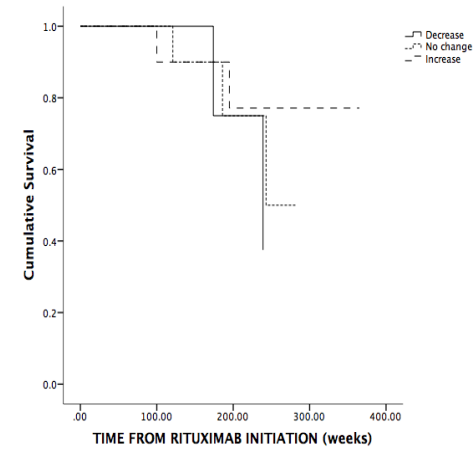
A **Log Rank p=0.756**

B **Log Rank p=0.913**

C **Log Rank p=0.121**

D **Log Rank p=0.474**

Kaplan-Meier analysis of the probability of hypogammaglobulinemia according to Trimethoprim-sulfamethoxazole (TMP-SMX) (A) and ciprofloxacin (B) antibiotic prophylaxis during rituximab maintenance

Figure 3

A   Log rank p=0.046

B   Log rank p=0.065