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Expression Of S Baff, R Baff, And B Cells (Cd19+) As Potential Biomarker In Predicting Respons Treatment After Pulse Dose Metyl Prednisolone In Severe Sistemic Lupus Erithematosus

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ABSTRACT

Background: B cell Activating Factors (BAFF) is a co stimulatory molecule that has become the target of recent SLE treatment. Pulse dose metyl prednisolone (MEP)has been widely used and is a standard protocol in management of severe and life threathening flare up. The aim of this study is to identify whethersoluble (S) BAFF along with its receptor,(R) BAFF and B Cells (CD19+) can become predictors of pulse dose metyl prednisolone (MEP) response treatment.

Method: Eighty subjects had been enrolled to this study. All subjects had SLE Disease Activity Index Score 2000 (SLEDAI 2K) score 12 or Lupus Nephritis Class 3 or 4 (WHO criteria) and decided to get pulse dose MEP. Prospective cohort study was conducted. All data as listed in SLEDAI 2k form were recorded before and after treatment. Enzyme-linked Immunoabsorbent Assay (ELISA) was used to measure S BAFF. Flowcytometry was used to measure BAFF Rand B cells (CD19+). Respons treatment was measured by SLEDAI 2K Responder Index (SRI) 50. Logistic Regression Analysis was used to identify the independent predictive factors for treatment response. Receiver Operating Characteristic (ROC) curve was used to find cut off point and Chi-sqare test to analyse proportion difference and Relative Risk (RR) to treatment response

Results: There were 80 subjects enrolled in this study, all with high disease activity (mean SLEDAI 2k 30.04 ±10.8).The differerence of SLEDAI 2K score before and after pulse dose MEP was 36%. SLEDAI 2K post MEP pulse > 36% of SLEDAI 2K pre treatment was considered SRI 36 Responder (SRI-36 R). SLEDAI 2K post pulse dose MEP < 36 % of SLEDAI 2K pre treatment was consider SRI -36 Non Responde (NR). Soluble BAFF 2459,47 pg/ml, R BAFF > 2817 MFI , and B cells (CD 19)> 29,2% have OR0.36; 2.17, and 2.16respectively but not statistically significant (p>0,05). Only R BAFF > 59,1 % have OR4.84with p value 0,03095% CI, in predicting SRI-36NR.

Conclusions: BAFF R> 59,1% is a biomarker predictor of SRI-36 NR in pulse dose MEP with OR4,84, p value 0,030(95%CI).

Keywords: *sBAFF, BAFF R, B cells (CD19), pulse dose, methyl prednisolone, predictors, biomarker, treatment response*

INTRODUCTION

B cells have important role in the pathogenesis of SLE by secreting autoantibodies, cytokines, and acting as Antigen Presenting Cells (APC). Autoreactive B cells activation is one of the hallmark of SLE. These are mainly mediated by a cytokine called BAFF, a member of tumour necrosis factor (TNF) family, had other name BLys (B lymphocyte stimulator)(Sellam et al., 2007). BAFF is expressed on the surface of monocytes, dendritic cells, neutrophils, stromal cells, and activated T cells. Founds in serum as a biologically active homotrimer. BAFF binds to its three receptors expressed by B cells in different lineages. B cell maturation antigen (BCMA) is expressed on transitional type 1 (T1) cells and plasma cells, whereas transmembrane activator and calcium modulator ligand interactor (TACI) and BAFF-R are expressed on transitional type 2/3 and mature B cells. BAFF R is upregulated by B cell receptor (BCR) ligation on mature B cells, and is expressed on resting memory B cells. BAFF R mediates most BAFF-dependent functions in the naïve B cells population (Ramanujam and Davidson, 2004).

Petri et al. showed, using multivariate analysis, that elevated baseline serum BAFF concentration (2 ng/ ml) was predictive of moderate-to-severe SLE flares in patients receiving the standard therapy, prednisone with anti malarial or immunosuppressive drugs(Petri et al., 2013). BAFF level was significantly higher during relaps as compared with disease remission (Carter, Isenberg and Ehrenstein, 2013). BAFF is a proven therapeutic target in SLE (Stohl, 2017).

Pulse dose methylprednisolone (MEP), 500-1000 mg/day for 1 to 3 days had been introduced in 1970's (Cathcart, 1976) and had good efficacy to overcome critical condition (Isenberg, 1982) such as in renal involvement, central nerve system, severe arthritis, pleuropericarditis, and thrombocytopenia (Funauchi and Yamagata, 2004). Elhefny et al found that pulse dose MEP isthe fastest immunosuppresionmethod in life or organ threathening SLE. It has good efficacy ini renal involvement as well as other severe manifestations like pulmonary hemorrhage or neuropsichyatry. Convensional treatment with iv MEP can decrease activity and complement activation in lupus nephritis, but some patients still experience progressive renal injury, even in that who had good respons, still have risk to relaps (Tsai et al., 2014).Since there are many studies report good correlation between s BAFF with SLEDAI 2k as disease activity parameter and the fact that disease activity can be overcome by pulse dose MEP, there are possibility that MEP have a role in decreasing S BAFF as a biomarker of disease activity. However, we lack objective parameters to predict respons of pulse dose MEP, besides respons to this treatmens is so various, and the safety profile for the increase risk of infection can be very high. We want to know whether S BAFF and its corresponding receptor BAFF R have correlation or even one of several predictors of pulse dose MEP treatment respons.

The study aims to identify SBAFF, R BAFF, and B cells (CD 19+) as a predictors of response treatment of pulse dose methylprednisolone in severe SLE.

METHODS

Study population

This was a cohort prospective study conducted from May 2021 to September 2022. Sample size was calculated using formula for prognostic reseach with cathegorical outcome data. Given the data for dependent variable was numeric, formula to be used was calculating mean difference between 2 groups. The formula was: n= 2 ((Z + Z)S: (x1-X2))2, = 5%; Z =1,96; =20%; Z= 0,84. The minimal sample size was 8. This research was a part of bigger study that have 5

predictors. The sample size used was following the rule of thumb formula for 5 predictors. Each predictor needs 15 subjects, the minimal sample size for 5 predictors was 75. Inclusion criteria were SLE patients meet ACR/EULAR 2019 criteria for SLE, SLEDAI 2K 12 or had class III or IV LN (WHO criteria) in induction phase treatment, decided to undergo pulse dose MEP 500 mg for 3 days, age range 15 to 50 years, and willing to participate in this study. Exclution criteria were patients with commobidities such as infection, diabetes melitus, malignancy, overlap disease, coronary heart disease, graves, in pregnancy.

Ethical considerations

Sampling was done from June 2021 to September 2022, after approved by Gadjah Mada University Faculty of Medicine, Nursing and Public Health ethics committee for human research in adherence to the Declaration of Helsinki (No: KE/FK/0359/EC/2021).

Assessment of SLE disease activity

Disease activities were assessed using SLEDAI 2K. Treatment respons were assessed using SLEDAI 2k responder index 50 form (SRI-50). SRI 50 form is the same form as SLEDAI 2k Form that show activity of 9 sytem organ with in 24 descriptors. The value ranges from 0-104. SRI 50 form identify 50 % improvement in each of the 24 descriptors in the SLEDAI 2k. The assigned scores derived by dividing the score of corresponding SLEDAI 2k by 2(Touma, Gladman and Urowitz, 2013)

Serum S BAFF examinations using Enzyme-linked immunosorbent assay (ELISA)

S BAFF was examined in PRODIA LABORATORIES, CAP certified, using Microplate Reader Biorad model 680Bio-rad Laboratories inc, CA, USA. Reagen kit used in this study was Quantikine® HS ELISA Human BAFF/BLyS/TNFSF13B(R&D Systems,Inc.,Minneapolis, USA) according to standard protocols. The results were measured in pg/ml.

Measurement of R BAFF and B cells (CD19) using flowcytometry

R BAFF and B cells(CD 19+) was examined in Clinical Pathology Laboratories of Gadjah Mada University Faculty of Medicine, Nursing and Public Health, ISO 17025 certified, using BD FACS Canto II 8 color Flowcytometry. Reagen used was PE anti human CD19 antibody (CD 19+) and CD 45 PerCP cy5.5 according to standard protocols. The results were measured in Mean Fluoresence Index (MFI), percentage of B cells CD 19+ to total lymphocyte , percentage of R BAFF to B cells CD 19+.

Statistical analysis

Statistical analysis was performed using the R software. Mean $\pm$ standard deviasion (SD) was used to describe normally distributed data. Median and interquartile range for skewed data. Frequencies of cathegorical data used percentage. Independent t and U test to analyse mean/median difference of variables. Paired t test to measure level of variables before and after pulse dose MEP. ROC curve analysis to find cut of point of each dependent variables toward outcome. Chi square analysis to measure proporsion difference and Odd Ratio(OR).

RESULT

Characteristics of study population

There were 80 patients eligible for this study. Female were 97.5%. Mean age was 28 $\pm$ 9.5 years. All subjects had severe flare, mean SLEDAI 2k score 30.04 $\pm$ 10.8. Duration of illness starting form first symptoms to study conducted were 33.94 $\pm$ 39.99 months. All baseline characteristics before pulse dose MEP were listed in table 1.

TABLE 1: Baseline characteristic of study population.

Variabel	mean(SD)	n (%)
Age (years)	28 (9.5)	
Sex Male Female		 2 (2.5) 78 (97.5)
SLEDAI 2K	30.04 (10.8)	
S BAFF (pg/ml)	2420.25 (2258.85)	
MFI BAFF R	4337.76 (3506.27)	
BAFF R (%)	78.63 (19.42)	
B cells CD 19+ (%)	19.35 (10.20)	
Duration of illness (months)	33.94 (39.99)	
ESR\mm/hour	69.79 (31.07)	
CRP (mean)mg/dl	22.6 (31.07)	
NLR	6.82 (5.49)	
SDI: 0 1 2		 58 (72.5) 16 (20) 6 (7.5)
Seizure		12 (15)
Psychosis		8 (10)
Organic Brain Syndrome Visual disturbance		20 (25) 4 (5)
Cranial nerve disorders		2 (2.5)
Lupus Headache CVA Vaskulitis		20 (25) 5 (6.3) 7 (8.8)
Arthritis Myositis Urin cast/	 5.16 (7.17)	51 (63.8) 12 (15) 47 (58.8)
Hematuria/hpf	218.5 (671.35)	59 (73.8)
Proteinuria (mg/g)	3648.86 (4233.83)	64 (80)
Leucocyturia /hpf	66.99 (177.77)	63 (78.8)
Rash		37 (46.3)
Alopecia		46 (57.5)
Oral ulcers		31 (38.8)
Pleurisy		21 (26.3)
Pericarditis		3 (3.8)
C3 (mg/dl)	51.55 (31.65)	73 (91.3)
C4 (mg/dl)	13.33 (11.49)	73 (91.3)
Anti Ds DNA (u/ml)	125.37 (80.42)	65 (81.3)
Fever		17 (21.3)
Leucocyte/	7257.63 (4484)	12 (15)
Thrombocyte/	193930.94 (104308.39)	16 (20)

Level of parameters disease activity before and after pulse dose MEP

After 3 days of pulse dose MEP, there were significantly decrease of SLEDAI 2K, S BAFF, urine cast, PCR, leucocyturia, anti ds DNA, C4 from 30.04 10.8; 2420.252258.85pg/ml; 5.16 7.17/hpf; 3648.864233.83mg/g; 66.99177.77/hpf; 131.86 94.6 U/ml; 13.33to19.268.86;1102.671180.69pg/ml;3.695.88/hpf;2812.423866.98mg/g;53.82149.93/hpf; 114.7983.88 U/ml; 11.199.67 mg/dl respectively. There were significantly increase of NLR, BAFF R, B cells CD 19+from 6,82 5.49; 78.6319.42 %; 19.3510.2% to 14.5517,56; 82.9618.98 %;

29.214.65 % respectively. There were increase of MFI BAFF R, hematuria, C3 from 4337.763506.27 MFI; 218.5671.35/hpf; 51.5531.65 mg/dl to 4992.2310442.99 MFI; 52.3530.76 mg/dl but not statistically significant (p>0.05) (Table2).

TABLE 2: Variable before and after pulse dose MEP

Variable	Pre, mean (SD)	Post,mean (SD)	p
SLEDAI 2K	30.04 (10.8)	19.26 (8.86)	0.00*
NLR	6.82 (5.49)	14.55 (17.56)	0.00*
S BAFF (pg/ml)	2420.25 (2258.85)	1102.67 (1180.69)	0.00*
BAFF R(%)	78.63(19.42)	82.96(18.98)	0.03*
BAFF R (MFI)	4337.76 (3506.27)	4992.23 (10442.99)	0.95
B cells CD19+(%)	19.35 (10.2)	29.2 (14.65)	0.00*
Cast urin/L	5.16 (7.17)	3.69 (5.88)	0.004*
Hematuria /hpf	218.5 (671.35)	261.54 (1442.98)	0.89
PCR (mg/g)	3648.86 (4233.83)	2812.42 (3866.98)	0.002*
Lekosituria /hpf	66.99 (177.77)	53.82 (149.93)	0.014*
C3 (mg/dl)	51.55 (31.65)	52.35 (30.76)	0.83
C4 (mg/dl)	13.33 (11.49)	11.19 (9.67)	0.00*
Ds DNA (U/ml)	131.86 (94.6)	114.79 (83.88)	0.00*

Mean decrease of SLEDAI-2k before and after pulse dose MEP was 35.89 % (36%). Treatment response were then categorized as above and below 36%. SLEDAI 2K post treatment above 36% of SLEDAI 2k of pre treatment was consider Non Responder (NR) while below were Responder (R). The ability to reach SLEDAI 2k post pulse dose MEP at least 36% from baseline SLEDAI 2k (SLEDAI 2k pre pulse dose MEP) were called SLEDAI 2k Responder Indeks 36 (SRI 36).

Level of each dependent variables between two groups (SRI-36 NR and SRI-36 R)

There were no significant median difference of S BAFF, MFI BAFF R, BAFF R(%), and B cells CD 19+ according to SRI 36 NR and R (p>0,05).

TABLE 3: Median difference of each variable according to SRI NR and R.

N0	Variabel	SRI-36 NR	SRI-36 R	p
1	BAFF S (pg/ml)			
	Median	1211.87	1827.73	0.29
2	MFI R BAFF			
	Median	3419	3125	0.29
3	R-BAFF (%)			
	Median	87.2	86.1	0.32
4	B cells CD19 (%)			
	Median	17.1	16.8	0.70

S BAFF, MFI BAFF R, BAFF R (%), B Cell CD 19+ (%) as predictors of SRI 36-NR

Numerical datas of independent variables were than changed to dichotomus datas to find the most effective cutoff point to distinguish its value above and below toward SRI 36 using Youden index.

TABLE 4: Determining cut off point of each dependent variable toward SRI 36 using ROC curve

	Youden index	Cut off	P value	Sensitivity	Specificity
S BAFF (pg/ml)	0.569	2459.47	0.303	76.7	45.9
MFI BAFF R	0,569	> 2817	0.569	72.1	45.9
BAFF R (%)	0,564	59.1	0.324	93	27
B Cell CD 19+(%)	0,525	>29.2	0.704	20.9	89.2

The ROC curve showed that cut off point for S BAFF, MFI BAFF R, BAFF R (%), and B Cell CD 19+toward SRI 36 were 2459.47 pg/ml; > 2817 MFI; 59.1%; >29.2 % respectively (Table 4). Chi square analysis showed than S BAFF ≤2459.47 pg/ml had RR 1.681 to get SRI 36 NR compared to S BAFF > 2459.47 pg/ml (p value = 0,057).This indicated that S BAFF ≤2459.47 pg/ml had higher risk to be non responder to MEP pulse, but not statistically significant. MFI BAFF R> 2817, BAFF R > 59.1, and B Cell CD 19+> 29.2 had RR 1.469, 2.587, 1.364 higher than MFI BAFF R ≤ 2817, BAFF R ≤ 59.1 %, B Cell CD 19+≤29.2 respectively (p value =0.150; 0.034; 0.358) (Table 5). This result showed that MFI BAFF R > 2817, BAFF R > 59.1, and B Cell CD 19+> 29.2 had higher risk to become non responder to MEP pulse, but only BAFF R> 59.1 % had statistically significant.

TABLE 5: Chi square analysis to measure mean difference of each variable according to SRI 36 NR and R

Variable	SRI-36 NR, N = 371	SRI-36 R, N = 431	Odds Ratio2	95% CI2,3	p-value2
BAFF S(pg/ml)			0.36	0.12, 1.03	0.037
Low	20 / 37 (54%)	33 / 43 (77%)			
High	17 / 37 (46%)	10 / 43 (23%)			
BAFF R (%)			4.84	1.11, 29.9	0.030
Low	10 / 37 (27%)	3 / 43 (7.0%)			
High	27 / 37 (73%)	40 / 43 (93%)			
BAFF R (MFI)			2.17	0.79, 6.18	0.11
Low	17 / 37 (46%)	12 / 43 (28%)			
High	20 / 37 (54%)	31 / 43 (72%)			
Bcell CD 19+(%)			2.16	0.54, 10.6	0.4
Low	33 / 37 (89%)	34 / 43 (79%)			
High	4 / 37 (11%)	9 / 43 (21%)			
1 n / N (%)					
2 Fisher's Exact Test for Count Data					
3 CI = Confidence Interval					

DISCUSSION

This study showed that mean s BAFF level before pulse dose MEP was 2420,25 ± 2258,85 pg/ml. This was higher than that found by Sellam et al 900 pg/ml. In that study median SLEDAI 2k was 4 indicating mild disease activity, compared to this study that all subjects had severe flare with mean SLEDAI 2k score 30,04 ±10,8(Sellam et al., 2007). Study by Duan et alhad similar result with this study that BAFF level in active group was 3367,22 ± 512,39 pg/ ml significantly higher than inactive SLE and healthy controle 2055 ±282,1and 899,7±63,41pg/ml respectively (p < 0,05)(Duan et al., 2016). Zaki et alalso found that BAFF level were higher ini severe compared to moderate and mild flare (1680 , 730, dan 300 pg/ml respectively),p < 0,05 (Zaki et al., 2019).

There were significantly increased level of BAFF R (%), B cells CD 19+, while not significantly increased level of MFI BAFF R after pulse dose MEP. Significant increase of B

cells CD 19+ before and after pulse dose MEP consistent with study conducted by Yan et al that prednison could significantly increase CD 19 expression on B cells as much as 8,25% and 11,33% in treatment groupsof 2,5 and 5 mg/ kg BB respectively and significantly decreased plasma cells precursors or even plasma cell themselves. This was due to the ability of prednison to hinder diferensiasion of B cells to plasma cells and subsequent autoantibodies(Yan et al., 2015) .

Level of BAFF R in active SLE inversely correlated with s BAFF level. All subjects in this study had high disease activities before pulse dose MEP, so BAFF R level were low. Mean BAFF R level were 78,63± 19,42 % and 4337,76 ± 3506,27 MFI. Study by Duan et al showed that BR3 level of active SLE were 49,77 ± 4,57 MFI significantly lower than inactive and healty controle group 67,96 ± 5,56 dan 85,79 ± 2,09 respectively, p value < 0,05 (Duan et al., 2016). Sellam et al showed than SLE patients with higher disease activity (SLEDAI ≥ 8), BAFF R level in B cells were lower than less active disease activity (SLEDAI <8) MFI 5,9 (3,5-8,5) and 10,1 (3,2-16,1) respectively , p=0,04 (Sellam et al., 2007). Similar result also found in study by Zaki et alalso that BAFF R level were lower in subjects with higher compared to moderate and mild disease activity 24,5; 49; dan 83,15 MFI respectively, p < 0,05 (Zaki et al., 2019).

We demonstrated that there were negatively correlation between s BAFF and BAFF R. It mean that the higher the level of S BAFF, the lower the level of BAFF R. Significant negative correlation only found between s BAFF and BAFF R (%), not with MFI R BAFF. There were positive correlation between BAFF R (%) and MFIBAFF R, this showed that the higher the level of BAFF R (%), the higher the level of MFI BAFF R. This indicated that level BAFF R (%) was better than MFI BAFF R in showing inverse correlation with S BAFF. S BAFF- BAFF R axis gives big implication in the patogenesis of SLE. Study by Zaki et alalso showed that s BAFF negatively correlated with BAFF R expression in B cell SLE patients (p<0,001). Decreased BAFF R level in the active group indicating the amount of BAFF R occupied by high level of S BAFF(Zaki et al., 2019).

Bivariate analysis between S BAFF and treatment respons measured by SRI-36 showed that median level of S BAFF before pulse dose MEP were lower in SRI 36-NR compared to SRI 36 – R groups, with no statistically significant. This could be explain by the notion that subject with lower S BAFF level, response to pulse dose MEP were lower than that with higher S BAFF level. Subjects with higher level of S BAFF response to pulse dose MEP were higher compared to that with lower level. This indicated that pulse dose MEP 500 mg for 3 days could lower high S BAFF level as much as 36 % of SLEDAI 2k before pulse dose with median level 1827,73 pg/ ml compared to 1211,87 pg/ml, even not statistically significant.Carter et al,reported that the expression of free available BAFF-R in blood B cells was decreased in SLE patients and associated more with disease activity than the serum BAFF level(Carter et al., 2005)

There were higher median level of MFIBAFF Rand BAFF R (%) in SRI 36 – NR compared to SRI 36 R with no statistically significant. Subjects with lower median MFI BAFF Rand BAFF R (%) level before pulse dose MEP (more active disease), response to pulse dose MEP 500 mg for 3 days were higher than group with higher median MFI BAFF R and BAFF R (%). This indicating that pulse dose MEP 500 mg for 3 days could increase lower MFIBAFF R and BAFF R (%) in severe SLE as much as 36 % of SLEDAI 2k pre pulse dose MEP.MFI BAFF R 3125 dan BAFF R 86,1% compsred to MFI BAFF R 3419 and BAFF R 87,2%, but not ststistically significant.

The lack of statistically significant differences in the median levels of MFI BAFF R between SRI 36-NR and SRI 36-R groups may suggest that the response to pulse dose MEP is not affected by the baseline levels of these parameters. However, subjects with lower median MFI BAFF R levels before pulse dose MEP (indicating more active disease) had a higher response to pulse dose MEP 500 mg for 3 days compared to the group with higher median MFI BAFF R levels. This result implies that pulse dose MEP can increase lower levels of MFI BAFF R in severe SLE as much as

36% of SLEDAI 2k before pulse dose MEP. Although the increase was not statistically significant, this finding suggests that pulse dose MEP may have a potential therapeutic effect on SLE patients with severe disease

There were proportion difference for SRI-36 NR according to BAFF R> 59 % and ≤ 59 % groups as much as 60,8 % and 41,4 % (p = 0,030) withOR 4.84. It means that subjects with BAFF R > 59,1% had risk of experiencing SRI 36 NR 2,587 higher than that with BAFF R ≤59,1%. This indicating that pulse dose MEP in SLE can increase BAFF R level ≤ 59 % or in other words had point of action in high level of s BAFFshowing that there were high proliferstion of autoreactive B cell to produce autoantibodies.While in subjects with higher BAFF R (Lower level of S BAFF) indicate that pulse dose MEP didn't have an action on lower S BAFF (normal level). The rational explanation was in normal or low level of S BAFF there were no active proliferation of B cells to produce autoantibodies. Other possible explanation was subjects in NR group that its proporsion were higher in group with lower SLEDAI 2K (≤ 24), there were protein other than BAFF so that this patients still categorized as high disease activity (SLEDAI 2K ≥ 12) that the level can not be lowered by pulse dose MEP, but maybe by targeted protein medication.

On the other hand, there were statistically significant differences in the median levels of BAFF R (%) between the SRI 36-NR and SRI 36-R groups. Subjects with BAFF R > 59,1% had a 4,84 -fold higher risk of experiencing SRI 36 NR than those with BAFF R 59,1%. It indicates that pulse dose MEP in SLE can increase BAFF R level 59%. In other words, pulse dose MEP has an impact at a high S-BAFF level, suggesting a high proliferation of autoreactive B cells to produce autoantibodies. Pulse dose MEP did not affect those who expressed higher BAFF-R since they had a lower level of S-BAFF. It is because there was no active proliferation of B cells to produce antibodies in those with normal or low S-BAFF levels. Another possible explanation was that subjects in the NR group (whose proportion was higher in the group with lower SLEDAI 2K) have proteins other than BAFF, thus still categorized as having high disease activity (SLEDAI 2K 12). These proteins cannot be affected by pulse dose MEP but preferably by targeted protein therapy.

CONCLUSIONS

R BAFF> 59,1% is a biomarker predictor of SRI-36 NR in pulse dose MEP with OR4.84, p value 0.030 (95%CI).

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