

1 **Impact of Premorbid Infection on Onset and Disease Activity of Rheumatoid Arthritis**

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20 **Abstract**

21 **Objective.** Infections have been implicated in rheumatoid arthritis (RA) development. However, the  
22 impact of premorbid infection on initiation and perpetuation of RA has not been well elucidated.  
23 Thus, we sought to conduct a large scale on-site survey to study whether premorbid infection may  
24 trigger RA and influence status of the disease.

25 **Methods.** Premorbid infectious events were collected in cohort of 902 RA patients from December  
26 2015 to June 2016. Type of infections prior to RA onset and its possible effects on disease status  
27 were analyzed.

28 **Result.** Three hundred and thirty-four out of 902 patients (37.03%) experienced infections within one  
29 month preceding RA onset. The most frequent infections were respiratory (16.08%), intestinal  
30 (11.09%) and urinary tract (9.87%) infection, respectively. The infection was associated with  
31 increased disease activity. Early onset was found in patients with urinary infection. High disease  
32 activity risk was increased in patients who pre-exposure to urinary infection (OR=3.813,  
33 95%CI=1.717-12.418) and upper respiratory infection (OR=2.475, 95%CI= 0.971-6.312).

34 **Conclusion.** Pre-exposure infections are associated with development of RA. Severe disease status  
35 of RA and persistent of active disease status are related to preceding infections.

36 **Keywords:** Premorbid infection, Rheumatoid Arthritis, RA onset, disease activity

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## 45 **Introduction**

46 Rheumatoid arthritis (RA) is a common autoimmune disease characterized by joint destruction and  
47 auto-antibodies production.[1] Many studies have demonstrated that infectious agents may contribute  
48 to the initiation or perpetuation of RA through a variety of mechanisms. Infection can cause a local  
49 inflammatory response. The innate immune system could also be affected by infectious agents and  
50 then cause RA onset, for instance, pathogen-associated molecular pattern receptors, especially the  
51 Toll-like receptors (TLRs) could release inflammatory mediators rapidly after recognizing some  
52 preserved structures in bacteria and other infectious agents [2].

53 Although a definite causative link between a specific infectious agent and the disease has not  
54 been established, several arguments support such a possibility. First, in the absence of a certain  
55 pathogen, the spectrum of microorganisms involved in triggering RA may include poly-microbial  
56 communities or the cumulative effect of bacterial or virus factors [3]. Secondly, infections didn't lead  
57 to RA in all cases, but initiate it in a certain subset of patients who was born with a genetic  
58 susceptibility [4-7]. Thirdly, some arthritis occurred based on pre-exposure to microorganism.  
59 Several animal models of arthritis are dependent on TLR2, TLR3, TLR4 or TLR9, for instance,  
60 rodents injected with streptococcal cell walls (TLR2 ligand) develop severe polyarticular arthritis  
61 and TLR4 ligand also play a role in passive K/BxN arthritis [8]. Many studies have shown that  
62 components derived from infectious agents can cause autoimmune reaction by molecular mimicry  
63 and other mechanisms. Epstein-Barr virus (EBV) is a polyclonal B lymphocyte activator which can  
64 increase the production of RF [4]. Oral pathogens may trigger the production of disease-specific  
65 autoantibodies and arthritis in susceptible individuals. It has been shown recently that RA is

66 associated with exposure to some microorganism such as *Aggregatibacter actinomyce-temcomitans*  
67 (Aa) [1].

68 In this study, we sought to conduct a large-scale survey to explore potential infectious agents  
69 which might initiate RA and the clinical consequence of this disease.

70

## 71 **METHODS**

72 **Patients** Survey results were collected from 902 RA patients admitted to the Department of  
73 Rheumatology and Immunology, People's Hospital, Peking University, between December 2015 and  
74 June 2016. All the studied patients fulfilled the American College of Rheumatology/European  
75 League Against Rheumatism Classification criteria for RA in 2010, and written informed consent  
76 was obtained.

77 The clinical data were recorded including tender and swollen 28-joint counts, general health on  
78 visual analog scales, erythrocyte sedimentation rate (ESR), Health Assessment Questionnaire  
79 (HAQ), 28-joint Disease Activity Score (DAS28) and the infectious agents one month before RA  
80 onset.

81 The questionnaire including age, sex, disease duration, age at symptom, smoking status, DAS28  
82 using the ESR at enrolment and treatments (one DMARD, more than one DMARDs, DMARDs plus  
83 low-dose glucocorticoid and bDMARDs). Only premorbid infectious agents of the RA patients were  
84 carefully recorded in this study.

85 **Statistical analyses** Analysis of covariance and multivariate logistic regression analysis were applied

86 to compare the disease activity in patients with or without prior infections. T test or ANOVA was  
87 used to analyze the data. The categorical variables were compared with chi-squared test. Multinomial  
88 logistic analysis was used to find risk factor which perhaps affected the current disease activity in RA  
89 patients. Data was expressed as mean  $\pm$  stand errors for continuous variables. The SPSS statistical  
90 package, version 23.0 was used for all statistical analyze, and p value less than 0.05 was considered  
91 statistically significant.

92

## 93 **Results**

### 94 **1. Prevalence of infections in RA**

95 Within one month prior to RA onset, 37.03% (334/902) patients experienced infections, and the most  
96 frequent sites were respiratory (16.08%), intestinal (11.09%) and urinary (9.87%), respectively  
97 (Table 1).

### 98 **2. Patients in severe disease status showed high prevalence of infections**

99 Four-hundred and ninety out of 902 RA patients with complete clinical data were analyzed in this  
100 study. These patients were divided into two groups based on DAS28 (DAS28<3.2 as group 1;  
101 DAS28 $\geq$ 3.2 as group 2). Compared with patients in group 2, patients in group 1 showed high  
102 prevalence of non-premorbid infection ( $\chi^2=18.193$  , P=0.000) (Table 2). Notably, patients with high  
103 disease activity suffered more pre-exposure of respiratory, intestinal and urinary infections (P=0.000,  
104 P=0.000, P=0.023; respectively) (Table 2). Besides, higher ESR and CRP were observed in patients  
105 with higher DASD28 scores (Table 3).

### 106 **3. Disease Activity was associated with premorbid infection.**

107 In our study, patients showed higher DAS28 in urinary (P=0.000) and respiratory (P=0.001) infection  
108 groups (Table4) before adjusting confounding factors such as the different therapies, age and  
109 smoking status which can affect disease activity.

110 One hundred and forty-five RA patients experienced respiratory tract infections one month prior  
111 to onset of the disease. Among these patients, 13.30% (120/902) patients showed upper respiratory  
112 tract infection while 2.77% (25/902) patients with lower respiratory tract infection. The number of  
113 tender and swollen joints (Fig 1A and B), HAQ scores (Fig 1D) and DAS 28 (Fig 1E) were higher in  
114 patients who had the respiratory tract infection compared with patients who had no infection before  
115 RA occurred. Furthermore, DAS28 was higher in respiratory infection group after adjusting for the  
116 age (P=0.002) and smoking (P=0.002) (Table4)

117 There were 89 patients with urinary infection who developed RA in one month before disease  
118 initiation. More deformed joints (Fig 1C) were found in patients who had premorbid urinary  
119 infection. The age at onset was younger in patients who had urinary infection. (Fig 1F) DAS28 was  
120 still higher in urinary infection group after adjusting for the therapy type (P=0.000) and smoking  
121 (P=0.002) group (Table4).

122 Intestinal infection occurred in 100 patients who developed RA. No difference was observed in  
123 these patients compared to patients with no infection. (Fig 1A-F) After adjusting age and smoking,  
124 DAS28 didn't show significant difference between intestinal infection group and no infection group  
125 (Table4).

#### 126 **4. Potential risk factors for high disease activity.**

127 The multinomial logistic regression was trained for predicting the disease activity with the factors

128 which showed statistical significance in single-factor analysis (Supplementary Table 2). These model  
129 parameters were for the low, moderate and high levels of disease activity, measured relative to the  
130 remission level (reference outcome). High disease activity risk was increased in patients who had  
131 urinary infection (OR=3.813,95%CI=1.717-12.418) (Figure 2), and upper respiratory infection  
132 (OR=2.475, 95%CI= 0.971-6.312) (Figure 2).

133

## 134 **Discussion**

135 There is increasing awareness that mucosal surfaces, including the gut and lungs, was sites of disease  
136 initiation in RA [8]. Recent studies showed that infectious agents including virus and bacteria  
137 infection had been associated with several kinds of autoimmune disease [7,10-12]. For instance,  
138 upper respiratory tract and other infections are well-known risk factors for multiple sclerosis [13].  
139 However, it was not clearly whether infectious agents play the causative role in the onset or outcome  
140 of autoimmune disease, this is mainly due to the lack of strictly perspective epidemiological study.  
141 And even in animal models, these relationships are complex and depend on the timing of exposure,  
142 antigen type and genetic background [14]. In our study, the age of disease onset was younger in  
143 patients who had urinary tract infection, which perhaps indicates that RA occurred earlier in patients  
144 with this pre-exposure infection and later in the other patients.

145 It has been certified that many virus can play a role in the production of auto-antibodies such as  
146 anti-cyclic citrullinated peptide [15]. Infections are known to cause or enhance autoimmunity  
147 through expansion of auto-reactive T-cell clones by molecular mimicry and enhanced antigen  
148 presentation [14]. The patients with infection events during the disease duration could have advanced

149 RA status [16]. To our knowledge, there was no study to prove the relationship between the  
150 premorbid infection history and onset or outcomes of RA in large populations. Here, we made the  
151 first report that analyzed this relationship in RA patients from outpatient of department of  
152 rheumatology and immunology in People's Hospital, Peking University.

153 There were many factors reflected the disease activity in RA, such as the number of tender or  
154 swollen joints, ESR, CRP and so on. Patients with respiratory tract infection had higher DAS28 and  
155 more swollen/tender joints. This probably because of respiratory tract infection was mainly caused  
156 by viruses. Acute viral infection in adults have long been suggested to induce transient autoimmune  
157 responses, including generation of autoantibody [7]. As reported in a recent study, Arleevskaya et al  
158 found that higher percentages of first-degree healthy relatives (HR) than health control (HC) had  
159 upper respiratory and urinary tract infections. During 10-year follow-up, 26 out of 251 (10.36%) HR  
160 subjects developed to RA, while no RA was found in HC group [4]. In our study, we found that  
161 9.87% (89/902) patients had pre-exposure of urinary tract infection and 13.30% (120/902) patients  
162 with upper respiratory infection. Besides, the patients with urinary infection were more likely to stay  
163 in disease activity stage and have more deformity joints. Moreover, the patients with respiratory  
164 infection had higher disease activity compared with no infection patients.

165 In fact, it is impossible to make a causal link between a specific pathogen and the disease. Our  
166 study has several limitations. First, because the study was done in a retrospective manner, the  
167 patients who had no complete clinical data were excluded from this study. Second, the number of the  
168 studied patients was not large enough to see the statistical difference in clinical features and odds  
169 ratio in lower respiratory tract infection subgroup patients. It may be due to this study group with



170 very few patients. Third, our studied patients may have selection bias because it was performed in a  
171 single university hospital. In order to determine the impact of premorbid infectious agents for RA  
172 outcome, the disease activity at RA onset and radiographic joint damage should be followed up in a  
173 larger prospective study.

174

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## 180 **【Reference】**

- 181 [1] Konig MF, Abusleme L, Reinholdt J, Palmer RJ, Teles RP, Sampson K, et al. *Aggregatibacter*  
182 *actinomycetemcomitans*-induced hypercitrullination links periodontal infection to autoimmunity in  
183 rheumatoid arthritis. *SicTransl Med*, 2016; 8(369):369ra176.
- 184 [2] Gray S. Firestein, Ralph C. Budd, Sherine E. Gabriel, et al. *Kelley's Textbook of Rheumatology*.  
185 Singapore: Elsevier PteLtd, 2015:1139-1142.
- 186 [3] Arleevskaya MI, Kravtsova OA, Lemerle J, Renaudineau Y, Tsibulkin AP. How Rheumatoid  
187 Arthritis Can Result from Provocation of the Immune System by Microorganisms and Virus. *Front*  
188 *Microbiol*. 2016; 17(7):1296.
- 189 [4] Arleevskaya, MI, Gabdoulkhakova, AG, Filina, YV, Miftakhova RR, Bredberg A, Tsybulkin AP.  
190 A transient peak of infections during onset of rheumatoid arthritis: a 10-year prospective cohort study.  
191 *BMJ Open*. 2014, 4(8):e005254.
- 192 [5] Leirisalo-Repo, M. Early arthritis and infection. *Curr Opin Rheumatol*. 2005; 17: 433-439.
- 193 [6] Benedek TG. The history of bacteriologic concepts of rheumatic fever and rheumatoid arthritis.  
194 *Semin Arthritis Rheum*. 2006; 36(2):109-23.
- 195 [7] Ori Barzilai, Maya Ram, Yehuda Shoenfeld. Viral infection can induce the production of  
196 autoantibodies. *Curr Opin Rheumatol*. 2007; 19(6):636-43.
- 197 [8] Choe JY, Crain B, Wu SR, Corr M. Interleukin 1 receptor dependence of serum transferred  
198 arthritis can be circumvented by toll-like receptor 4 signaling. *J Exp Med*, 2003; 197:537.
- 199 [9] Liu Y, Mu R, Gao YP, Dong J, Zhu L, Ma Y, et al. A cytomegalovirus peptide-specific antibody

200 alters natural killer cell homeostasis and is shared in several autoimmune diseases. *Cell Host*  
201 *Microbe*. 2016; 19(3):400-8.

202 [10] Brusca SB, Abramson SB, Scher JU. Microbiome and mucosal inflammation as extra-articular  
203 triggers for rheumatoid arthritis and autoimmunity. *Curr Opin Rheumatol*. 2014; 26:101–107.

204 [11] Haleniusm A, Henge H. Human cytomegalovirus and autoimmune disease. *Biomed Res Int*.  
205 2014:472978. doi: 10.1155/2014/472978. Epub 2014 Apr 29

206 [12] IgoeA, Scofield RH. Autoimmunity and infection in Sjögren's syndrome. *Curr Opin*  
207 *Rheumatol*.2013; 25(4):480-7.

208 [13] Bulijevac D, Flach HZ, Hop WC, Hijdra D, Laman JD, Savelkoul HF, et al. Prospective study  
209 on the relationship between infections and multiple sclerosis exacerbations. *Brain*. 2002; 125(pt  
210 5):952-60.

211 [14] MünzC, Lünemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or  
212 triggered by autoimmunity? *Nat Rev Immunol*. 2009; 9(4): 246-258.

213 [15] Costenbader KH, Karlson EW. Epstein-Barr virus and rheumatoid arthritis: is there a link?  
214 *Arthritis Res Ther*. 2006;8:204

215 [16] Iguchi-Hashimoto M, Hashimoto M, Fujii T, Hamaguchi M, Furu M, Ishikawa M, et al. The  
216 association between serious infection and disease outcome in patients with rheumatoid arthritis. *Clin*  
217 *Rheumatol*. 2016; 35(1):213-8.

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**Table 1 The type of premorbid infections in RA patients**

Infection types	Cases	Percentage (%)
No infection	568	62.97
Respiratory	145	16.08
Upper	120	13.30
Lower	25	2.77
Intestinal	100	11.09
Urinary	89	9.87

**Table 2 Prevalence of infection in RA patients with different disease activity**

Infection types	Group 1 (n=244)	Group 2 (n=246)	$\chi^2$	P value
	DAS28<3.2 (n, %)	DAS28≥3.2 (n, %)		
No infection	201(82.4)	161(65.4)	18.193	0.000
Respiratory	20(8.2)	39(15.9)	30.384	0.000
Intestinal	10(4.1)	17(16.9)	125.390	0.000
Urinary	13(5.3)	29(11.8)	7.518	0.023

**Table 3 Clinical characteristics and demographics of RA patients**

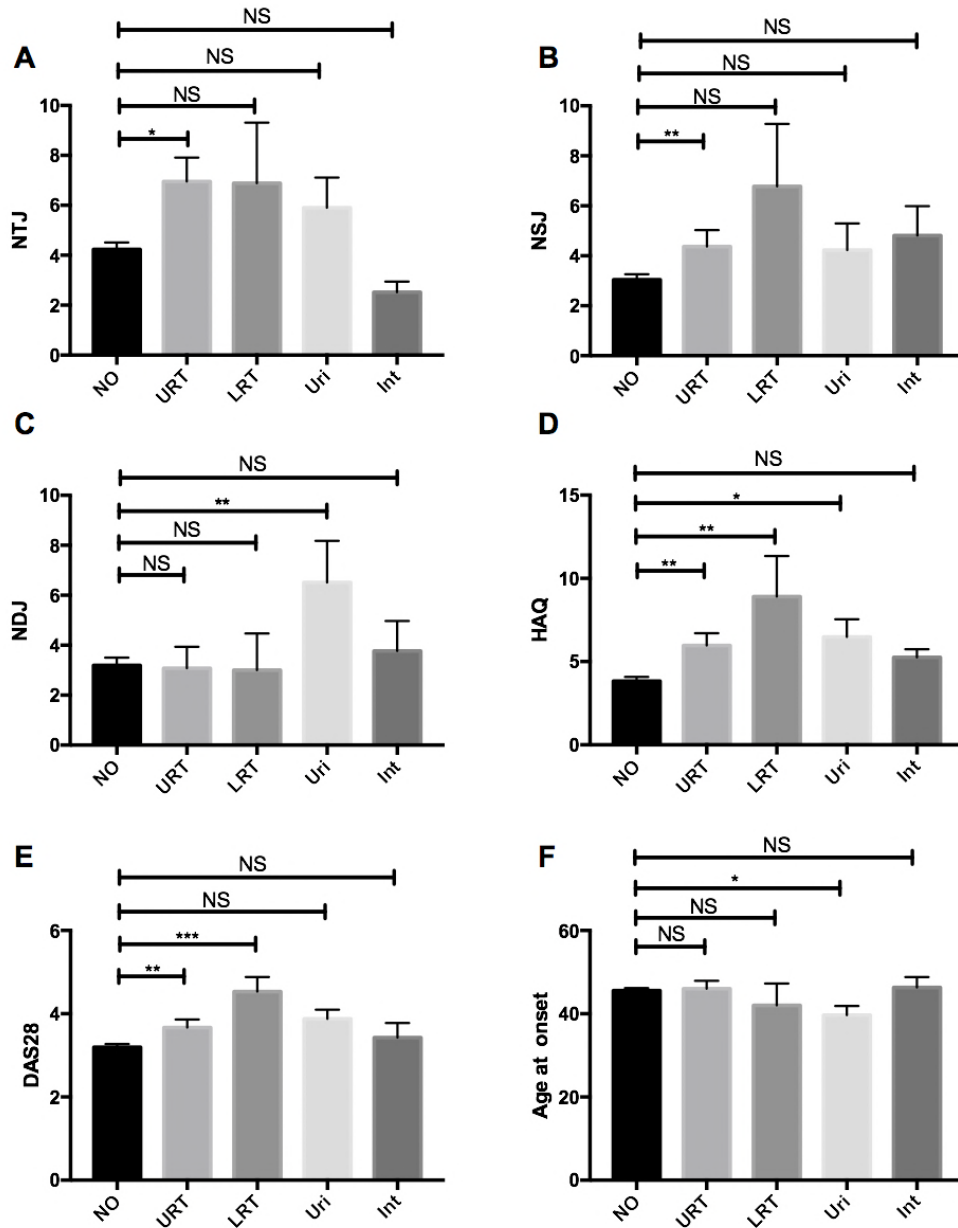
Characteristic	Group 1 (DAS28<3.2) (n=244)	Group 2 (DAS28≥3.2) (n=294)	Statistic	P value	
Male <sup>c</sup> , n (%)	50 (20.5)	50 (20.3)	4.601	0.100	
Age <sup>a</sup> (years)	54±14	55±13	-0.281	0.779	
Disease duration <sup>b</sup> (years)	3 (2, 5)	8 (3, 22.5)	-0.953	0.340	
Age at diagnosis <sup>b</sup> (years)	44±14	45±15	-0.781	0.435	
ESR <sup>b</sup> (mm/H)	11 (7, 18)	33 (19, 55)	-13.337	0.000	
CRP <sup>b</sup> (mg/L)	2.68 (1.44, 4.87)	9.58 (3.31, 23.19)	-10.531	0.000	
Anti-CCP negative <sup>c</sup> , n (%)	37 (37/223, 16.6%)	36 (36/154, 23.4%)	2.686	0.101	
Anti-CCP antibody <sup>b</sup> (U/L)	167.53 (57.2, 224.14)	165 (38.24, 225.21)	-0.419	0.675	
HAQ <sup>b</sup>	1 (0, 3)	5 (1, 12)	-10.013	0.000	
Smoking Status <sup>c</sup>	Never smokers	142 (142/209, 67.9)	157 (157/215, 73.0)	1.350	0.509
	Passive smokers	23 (23/209, 11.0)	19 (19/215, 8.8)		
	Active smokers	44 (44/209, 21.1)	39 (39/215, 18.1)		
Current Treatment <sup>c</sup>	One DMARD	56 (56/222, 25.2)	38 (38/203, 18.7)	15.904	0.001
	More than one DMARD	138 (138/222, 62.2)	109 (109/203, 53.7)		
	DMARDs + glucocorticoid	14 (14/222, 6.3)	23 (23/203, 11.3)		
	bDMARDs	14 (14/222, 6.3)	33 (33/203, 16.3)		

(ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; anti-CCP, anti-citrullinated peptide antibodies; a, Data is described as mean ±SD, analysis with t-test; b, Data are reported as median with top and bottom quartile, nonparametric test is used for analysis; c, Chi-squared testis used.)

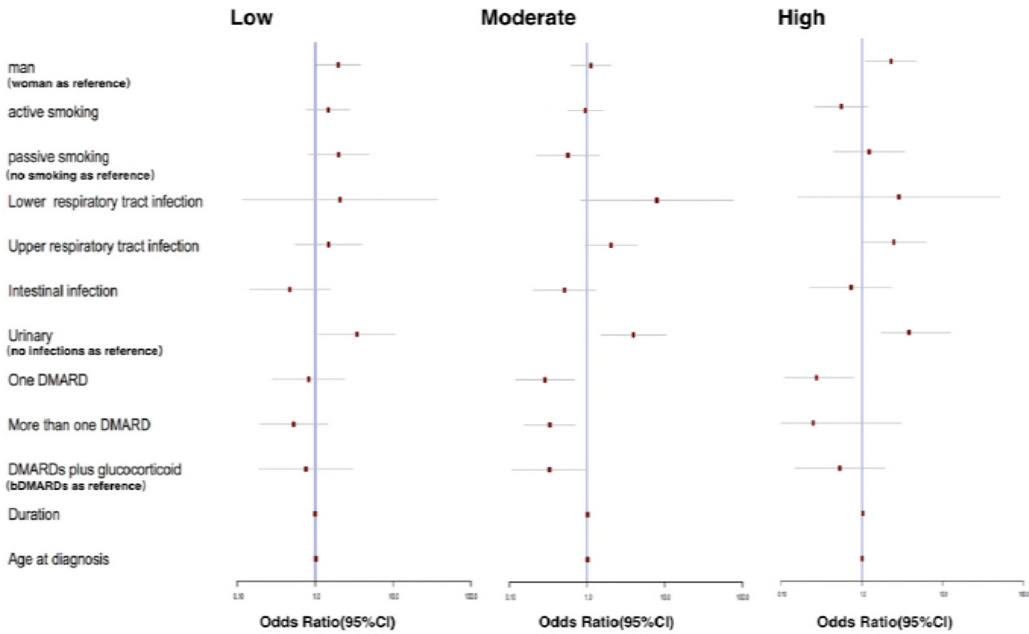
**Table 4 Differences of DAS28 between infectious groups and no infection group**

	No-infection	Urinary	Respiratory	Intestinal
Before adjusted	3.25±0.07	3.97±0.19 <sup>△</sup>	3.78±0.17 <sup>△</sup>	3.56±0.26
Adjusting for confounding factors				
Therapy <sup>a</sup>	3.20±0.07	3.91±0.20 <sup>△</sup>	—	—
Age <sup>a</sup>	3.26±1.41	—	3.80±1.450 <sup>*</sup>	3.58±1.50
Smoking <sup>a</sup>	3.24±1.38	3.95±1.56 <sup>*</sup>	3.82±1.50 <sup>*</sup>	3.62±1.51

Analysis of covariance was applied for adjusting confounding factors; a: Adjusted for therapy; b: Adjusted for age; c: Adjusted for smoking; —: Cannot be adjusted because of having an interaction effect compared with no-infection; Analysis of covariance was used between no-infection group and other infectious groups, \* : P<0.05 <sup>△</sup>: P<0.001.



**Fig1. Associations of disease activity and sites of infection.** These patients were categorical into subgroups including no infection (n=568), upper respiratory tract infection (n=120), low respiratory tract infection (n=25), urinary infection (n=89) or intestinal infection (n=100). Comparisons between groups were performed using the t-test or nonparametric test. The numbers of tender (A) and swollen (B) joints, HAQ (D) and DAS28 (E) were higher among respiratory tract infection, and number of deformity joints (C) was higher in urinary infection group. (F) Age at onset was younger in urinary infection group than other infection groups. (NO, no infection; URT, upper respiratory tract; LRT, lower respiratory tract; Uri, Urinary; Int, Intestinal)(\*: $P < 0.05$ ; \*\*: $P < 0.01$ ; \*\*\*: $P < 0.001$ )



Error bars indicate 95% confidence intervals.

**Figure2. Multinomial Logistic regression for the potential risk factors for high disease activity.**  
 (Remission as reference)