Background/Purpose: RA is an autoimmune, inflammatory and chronic disease which aetiology is unknown. It presents different autoantibodies such as RF and ACPA. A population of CD4 T cells expressing CXCR5, Bcl6, PD-1, ICOS, CD40L and IL-21, named Follicular helper T cells (Tfh), collaborates with B cells to produce antibodies. Increased levels of peripheral blood Tfh cells have been implicated in the development of systemic autoimmunity. Differential expression of CXCR3 and CCR6 whitin $CD4^+CXCR5^+$ T cells defines three mayor subset: $CXCR3^+CCR6^-$ (Tfh1), $CXCR3^-CCR6^-$ (Tfh2) and $CXCR3^-CCR6^+$ (Tfh17). The aim is to ascertain if differents subsets of $CD4^+CXCR5^+$ T cells are alterated in RA patients and if their percentages correlate with disease activity.

Methods: In this study participated RA patients (n=24), healthy controls (HC) (n=22) and undifferentiated arthritis (UA) patients (n=16) (Table 1). Percentage of $CD4^+CXCR5^+$ T cells and their subsets $CXCR3^+CCR6^-$, $CXCR3^-CCR6^-$ and $CXCR3^-$ $CCR6^+$ from PBMCs were analysed by flow cytometry. Pearson or Spearman correlation coefficients were used for statistics.

Results: Figure 1 shows flow cytometry analysis. No differences were found in the % of $CD4^+CXCR5^+$ T cells between RA vs HC or RA vs UA (mean±SD, RA 12,89±7,73; HC 10,48±3,9; UA 11,71±5,04). Either in the % of Tfh1 (12,75 ± 9,72; 11,22 ± 7,48; 12,81 ± 6,13), or Tfh2 (32,66 ± 11,46; 39,53 ± 12,12; 27,56 ± 11,25), or Tfh17 subsets (37,94 ± 11,34; 40,79 ± 8,17; 37,34 ± 7,16) between previous groups (Figure 2). There was not correlation between $CD4^+CXCR5^+$ T cells (r=-0,19 *p*=0,37), or Tfh1 (r=0,09 *p*=0,68), or Tfh2 (r=0,36 *p*=0,09), or Tfh17 (r=-0,20 *p*=0,35) vs DAS-28, like either between each subset and ESR (r=-0,18 *p*=0,39, r=-0,08 *p*=0,71, r=-0,01 *p*=0,97, r=-0,25 *p*=0,23, respectively). Unexpectedly, there was positive correlation between Tfh17 cells and CRP r=0,47 *p*=0,021. Finally, there was not correlation between $CD4^+CXCR5^+$ T cells vs mutated citrullinated vimentin (MCV) r= 0,38 *p*=0,07, either between Tfh1, Tfh2 and Tfh17 subsets vs MCV(r=-0,04 *p*=0,84, r=-0,14 *p*=0,51, r=-0,19 *p*=0,37, respectively) or all of them vs RF (r=0,30 *p*=0,15, r=-0,18 *p*=0,39, r= -0,15 *p*=0,46, r=0,01 *p*=0,98, respectively).

Conclusion: In concordance with our results, $CD4^+CXCR5^+$ T cells and their subsets would not be involved in the RA development.

TABLE 1. Summary of Patients and Donors in the Study

	RA (n=24)	HC (n=22)	UA (n=16)	RA vs HC *p* value	RA vs UA *p* value
Sex, F/M	**21/3**	**19/3**	**13/3**	**0,77#**	**0,93#**
Age,* years	**51 ± 10**	**49 ± 10**	**52 ± 11**	**>0,05**	**>0,05**
WBC,* n°.10⁹/L	6,98 ± 1,85	7,23 ± 1,96	6,84 ± 1,74	>0,05&	>0,05 &
Hgb,* g/L	**12,6 ± 1,91**	**12,6 ± 0,98**	**12,96 ±1,07**	**>0,05$**	**>0,05$**
Plat* n°.10⁹/L	255 ± 80	254 ± 41	249 ± 59	>0,05$	>0,05$
ESR,*mm/h	25 ± 21	9 ± 6	14 ± 12	**<0,01$**	>0,05$
CRP,* mg/L	18 ±14	9 ±2	14 ± 8	**<0,01$**	>0,05$
RF +, n (%)	19 (79)	0 (0)	0 (0)	**<0,0001#**	**<0,0001#**
MCV, UI/L**	75,0(7,7-530,0)	2,8(2,3-5,3)	2,9(2,5-3,8)	**<0,001$**	**<0,001$**
IgG*, mg%	1310 ± 338	1325 ± 257	1212 ± 336	>0,05#	>0,05#
IgM*, mg%	220 ± 88	169 ± 55	179 ± 60	**<0,05#**	>0,05#
IgA*, mg%	363 ± 126	313 ± 80	261 ± 114	>0,05#	**<0,05#**
C3*, mg%	112 ± 33	119 ± 26	130 ± 30	>0,05#	>0,05#
C4*, mg%	24 ± 9	26 ± 7	31 ± 8	>0,05#	**<0,05#**
DAS-28*	**5,16 ± 1,35**	----------	----------	----------	----------

*Value given in mean ± SD
** Value given in median and interquartile range (P_{25-75})
# Chi-square test
& One- way ANOVA test (post test Bonferroni)
$ Kruskal- Wallis test (post test Dunn)
Satatistically significant p values (p<0,05) are shown in bold
RA: Rheumatoid Arthritis, HC: Healthy Control, UA: Undifferentiated Arthritis, WBC: White Blood Cell, Hgb: Hemoglobin, Plat: Platelets, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein,
RF: Rheumatoid Factor, MCV: anti-Mutated Citrullinated Vimentin, Ig: Immunoglobulin. DAS-28: Disease Activity Score in twenty-eight joints

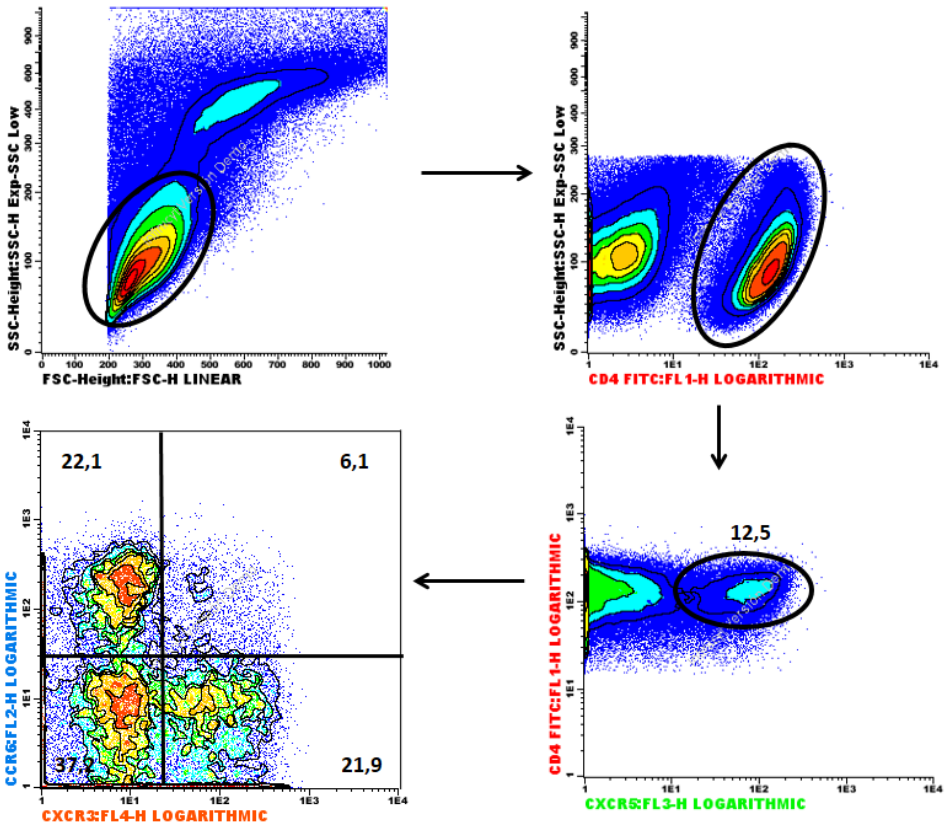

Figure 1. Density contour graph 2D showing gate strategy of a representative experiment from a patient with RA

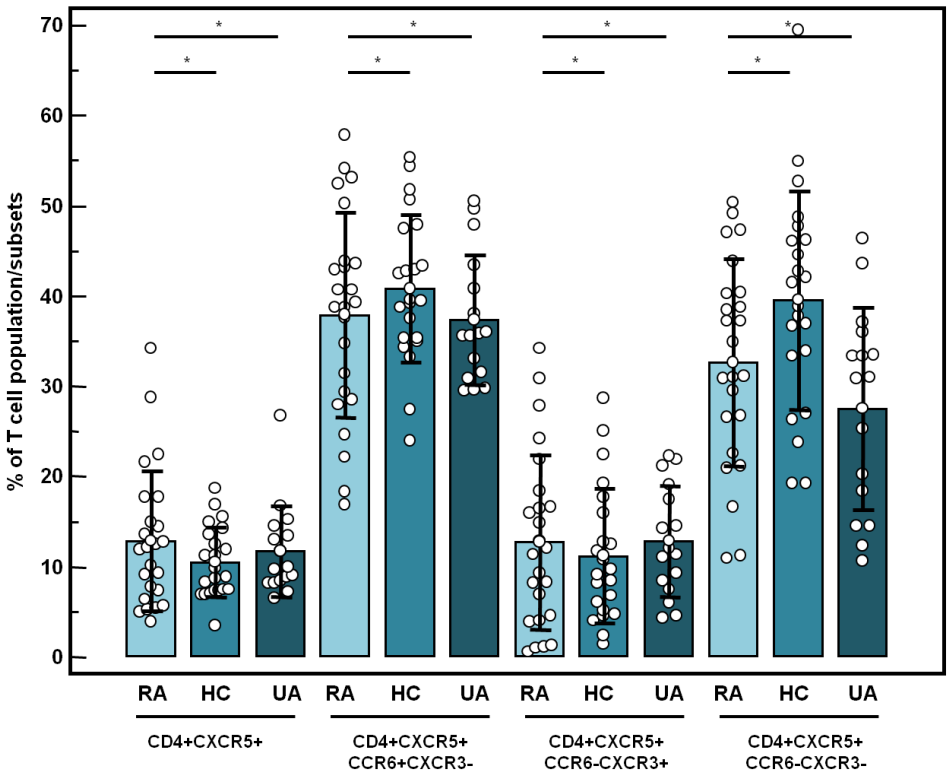

Figure 2. Percentages of CD4+CXCR5+ T cell population and each subset in PBMCs from RA patients, Healthy Controls (HC) and Undifferentiated Arthritis (UA) patients. One-way ANOVA test and Bonferroni post test , * p>0,05