Progenitor mast cells and tryptase in Q fever

Soraya Mezouar, Victor Morel, Laury Leveille, Noémie Resseguier, Céline Chartier, Didier Raoult, Jean-Louis Mege, Joana Vitte

Progenitor mast cells and tryptase in Q fever

Soraya Mezouar[1*], Victor Morel[1], Laury Leveille[1], Noémie Resseguier[2], Céline Chartier[3], Didier Raoult[1], Jean-Louis Mege[1,3] and Joana Vitte[1,3]

[1]Aix-Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Marseille, France
[2]Department of Epidemiology and Health Economics, Aix-Marseille Univ, Marseille, France
[3]APHM, IHU Méditerranée Infection, UF Immunologie, Marseille, France

[*]Corresponding author :
Dr. Soraya Mezouar
IHU Méditerranée Infection
19-21 Boulevard Jean Moulin
13385 Marseille, France
Phone: (+33) 4 13 73 20 51
Fax : (+33) 4 13 73 20 52
E-mail: soraya.mezouar@univ-amu.fr

Abstract

Q fever is an infectious disease due to *Coxiella burnetii*. Following a primary-infection, *C. burnetii* may persist in some patients, leading to endocarditis and vascular infections. Mast cells (MCs), known for their role in allergic diseases, innate immunity and cardiac function, are produced by bone marrow, circulate as progenitors in the bloodstream and reach tissues for their maturation and activation. The latter may be estimated by measuring serum tryptase levels. We wondered if MC progenitors and tryptase were affected in Q fever. We showed a decrease in MC progenitor count in Q fever patients whereas serum tryptase levels were increased. ~~Interestingly, counts MC progenitors and levels of serum tryptase were correlated in Q fever patients with valvular lesions including Q fever endocarditis.~~ Taken together, our results show alterations of MC numbers and activity in Q fever patients, suggesting that MC are involved in Q fever pathophysiology.

Keywords: Mast cells progenitors, tryptase, Q fever, *Coxiella burnetii*, flow cytometry

1. Introduction

Q fever is an infectious disease due to the intracellular bacterium *Coxiella burnetii*. Following primary-infection that is symptomatic in some patients, the infection may become persistent in specific contexts such as immunodeficiency, valvulopathy or vascular disease. The manifestations of persistent Q fever consist of endocarditis and vascular infections [1]. The evolution of Q fever is largely determined by anti-*C. burnetii* immune response [1]. This latter consists of an inappropriate inflammatory response and decreased counts of lymphocytes [2], monocytes [3], dendritic cells [4], and plasmacytoid dendritic cells in Q fever patients [5]. It is likely that other innate immune cells including mast cells (MCs) are involved in the pathophysiology of Q fever. MCs leave the bone marrow as progenitors, pass through the bloodstream and complete their maturation in target tissues [6]. MCs are key players in both inflammatory and immune responses, in addition to their well-known role during immediate hypersensitivity reactions [7]. MCs also contribute to cardiac functions and are involved in cardiovascular diseases [8]. Mast cell progenitors may be found in the bloodstream and are identified as $CD34^+$ cells expressing CD117 (c-kit) in association with the high affinity immunoglobulin (Ig) E receptor (FcεRI) [9]. During infection, MC progenitors are recruited to infected tissues where they mature and get activated, contributing to host defense mechanisms against microorganisms [10]. The activation status of tissue MCs can be estimated by the determination of serum baseline tryptase (sbT) [11]. *C. burnetii* has a strong tropism for tissues rich in mast cells (MCs) such as adipose tissue [12], bone-marrow [13,14] or lung [15]. However, the role of MCs in Q fever is unknown.

We wondered if MCs are involved in the pathophysiology of Q fever. The main purpose of this study was to investigate the MC progenitor population in Q fever patients. Using flow cytometry, we report here that circulating MC progenitors were decreased whereas sbT was increased in Q fever patients compared to healthy donors. ~~We also reported a correlation between the decrease of circulating MC progenitors and the increase in sbT in Q fever patients with valvular disease.~~ Taken together, these results suggest a role of MCs in Q fever pathophysiology.

2. Materials and methods

2.1 Patients and controls

We included 23 healthy blood donors and 22 patients with Q fever. Q fever patients belonged to clinical subsets of acute (n = 10) and persistent Q fever (endocarditis group, n = 12). This study was conducted with the approval of the Ethics Committee of Aix-Marseille University and written consent of each patient. Patients consisted of 6 women and 16 men, median age 63, range 35 - 89 years. Controls consisted of 11 women and 12 men, median age 41, range 24 – 65 years. The diagnosis of acute and persistent Q fever was performed according to recently updated criteria [1]. Briefly, patients with acute Q fever were diagnosed by the presence of fever and/or hepatitis and/or pneumonia with serological criteria (IgG >200 and IgM>50 against *C. burnetii* phase II or seroconversion) [1]. Persistent Q fever was diagnosed on the presence of hepatitis, endocarditis or vascular infection and IgG >800 against *C. burnetii* phase I. As additional controls we included 15 patients with acute, non-Q fever, native valve infective endocarditis. Patients consisted of 6 women and 9 men, median age 61, range 21 – 81 years. The diagnosis of acute infective endocarditis was performed according to modified Duke criteria [16]. Valvular surgery was performed in all patients as valvular repair or valvular replacement. A microbiological identification was obtained in all patients. *Staphylococcus aureus* and *S. lugdunensis* were found in 4 and 1 patient respectively. *Enterococcus faecalis* was found in 3 patients. *Streptococcus anginosus*, *S. mitis* and *S. infarius* was respectively found in 1 patient. The other microorganisms recovered were *Gemella sanguinis*, *Haemophilius influenzae*, *Escherichia coli* and *Bartonella quintana*.

2.2 Cell isolation

Blood was collected into EDTA tubes. Peripheral blood mononuclear cells (PBMCs) from healthy donors and patients were isolated after centrifugation through Ficoll cushion and suspended in RPMI 1640 containing 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Life Technologies, Courtaboeuf, France) as previously described [3].

2.3 Serum baseline tryptase quantification

Serum baseline tryptase levels were measured with the immunofluorescent enzyme assay for α- and β-tryptase isoforms (ImmunoCAP, Thermo Fisher, Uppsala, Sweden).

2.4 Flow cytometry

Mast cell progenitors were identified by staining with anti-CD34 (Beckman Coulter, Nyon, Switzerland), anti-CD117 (c-Kit receptor, CD117-APC, Beckman Coulter) and anti-IgE (FcεRI, anti-IgE-PE, Bühlmann, Schönenbuch, Switzerland) antibodies (Abs). Cytometry experiments were performed with a Canto II flow cytometer (Becton Dickinson, Le Pont de Claix, France). Fifty thousand events were acquired and analyzed with FACS Diva software (Becton Dickinson Bioscience).

2.5 Statistical analysis

Results were expressed as median and range. Statistical analysis was performed using the Mann-Whitney *U* test. Adjusted p-values were provided in order to take into account the multiplicity of comparisons within each analysis. The Benjamini and Hochberg method was used, thus controlling the false discovery rate. Statistical significance threshold was set at $p < 0.05$.

3. Results

3.1 Circulating MC progenitors were specifically decreased in Q fever patients

The expression of CD117, surface IgE and CD34 was assessed by flow cytometry to identify MC progenitors, as previously reported [9]. We found that approximately 1% of total PBMCs were $CD117^+/IgE^+$, and 0.003 % were $CD34^+$ MC progenitors ranging from 0.001 % to 0.012 % of total PBMCs (**Figure 1A**). Then we wondered if this subset of circulating cells was modulated in Q fever. We found that the percentage of MC progenitors was significantly decreased in patients with Q fever (p = 0.001) (**Figure 1B**) whereas no differences were observed between acute and endocarditis group (p = 0.8836) (**Figure 1C**). In addition, the percentage of MC progenitors was not altered in patients with bacterial infection other than *C. burnetii* (p = 0.1515). These results showed that MC progenitors were specifically decreased in Q fever patients independently of their clinical form.

3.2 Serum baseline tryptase was specifically increased in patient infected with *C. burnetii*

We therefore investigated sbT in Q fever patients. As depicted in **figure 2A**, sbT was higher in Q fever patients than in controls (6.18 ± 2.87 µg/L *versus* 3.77 ± 1.85, p = 0.0448). Interestingly, compared to controls this increase is specifically found in Q fever patients because other infections did not induce modification in sbT (**Figure 2A**). In addition, no differences were observed in acute and endocarditis groups (p = 0.4700). These results showed a specific increase of sbT in Q fever. ~~Moreover, we found a correlation between the number of MC progenitors and sbT levels in one subgroup of Q fever patients, namely in Q fever patients with valvular lesions including Q fever endocarditis (R = 0.8240, p = 0.0034) (**Figure 2B**). These results suggested that this association was a marker of vascular involvement in Q fever.~~

4. Discussion

In this study, we measured the frequency of MC precursors in blood from healthy individuals and patients with Q fever. The identification was based on the assessment of the co-expression of IgE, CD117 and CD34. This approach is necessary because each maker is incompletely specific of MC progenitors. IgE expression identifies mostly MCs and basophils, but monocytes from atopic patients can also bear surface IgE [17]. Although CD117 is widely considered as a specific MC surface marker, it is also expressed by basophils, myeloid dendritic cells, TCRα/β$^+$ T cells, B cells and NK cells [18]. We found that MC progenitors represent a minor population in blood from healthy individuals. This is in agreement with previous studies [6,9].

This study reported for the first time the decrease in circulating MC progenitors in Q fever patients. This result has to be related to previous reports in which we found a decrease in circulating lymphocytes [2], monocytes [3], dendritic cells [4] and plasmacytoid dendritic cells counts [5]. While the decrease in circulating immune cells occurred mainly in Q fever patients with endocarditis, here we did not observe differences in the number of MC progenitors between acute and persistent Q fever. Decreased numbers of progenitors might be due to impaired bone marrow production, or increased recruitment of progenitors into *C. burnetii*-infected tissues. It has been reported that the numbers of MC increased in tissue-specific due to the maturation of MC progenitors in human diseases [6,19]. Alternatively, the decrease in MC progenitors may be due to their death. The interleukin (IL)-10 is known to induce MC apoptosis [20] and is increased in persistent Q fever [21].

The second major observation was the increase in serum tryptase in patients with Q fever independently of the clinical presentation. Circulating tryptase originates mainly from mature MCs and in minute amounts from MC-committed progenitors [22]. Apart from acute MC degranulation, which was not relevant for the patients included in this study, an increase in tryptase levels has been reported in association with a poorer clinical condition or prognosis in cardiovascular diseases [23]. ~~Indeed, in our hands, the increase in tryptase levels was correlated in Q fever patients with valvular lesions including Q endocarditis. This association is reminiscent of the abundant presence of MCs in cardiovascular tissues. The cardiac tropism of MC and the variation of the sbT is well documented in particular in cardiovascular diseases such as aortic stenosis and myocardial infarction [24,25].~~

In summary, we show that Q fever patients display less circulating MC progenitors but higher levels of sbT. ~~The decrease in MC progenitors is correlated with sbT in Q fever~~

~~patients with valvular lesions including endocarditis.~~ These results suggest MCs take part to pathophysiology of Q fever.

Acknowledgments

We thank Dr. Christian Capo and Dr. Yassina Bechah for their help and advice during the preparation of the manuscript.

Formatting of funding sources

Soraya Mezouar was supported by a "Fondation pour la Recherche Médicale" (FRM) postdoctoral fellowship (SPF20151234951). This work was supported by the French Government under the "Investissements d'avenir" (Investments for the Future) program managed by the "Agence Nationale de la Recherche" (reference: Méditerranée Infection 10-IAHU-03).

Author contributions

S.M and J.V conceived and designed the experiments. S.M, V.M, C.C and L.L performed experiments and analyzed the data. N.R performed statistical analysis. S.M, D.R, J.L.M and J.V wrote the paper.

Declaration of interest

The authors declare no competing interests.

References

[1] C. Eldin, C. Mélenotte, O. Mediannikov, E. Ghigo, M. Million, S. Edouard, J.-L. Mege, M. Maurin, D. Raoult, From Q Fever to Coxiella burnetii Infection: a Paradigm Change, Clin. Microbiol. Rev. 30 (2017) 115–190. doi:10.1128/CMR.00045-16.

[2] C. Layez, C. Brunet, C. Lépolard, E. Ghigo, C. Capo, D. Raoult, J.-L. Mege, Foxp3(+)CD4(+)CD25(+) regulatory T cells are increased in patients with Coxiella burnetii endocarditis, FEMS Immunol. Med. Microbiol. 64 (2012) 137–139. doi:10.1111/j.1574-695X.2011.00902.x.

[3] M.B. Ka, F. Gondois-Rey, C. Capo, J. Textoris, M. Million, D. Raoult, D. Olive, J.-L. Mege, Imbalance of circulating monocyte subsets and PD-1 dysregulation in Q fever endocarditis: the role of IL-10 in PD-1 modulation, PloS One. 9 (2014) e107533. doi:10.1371/journal.pone.0107533.

[4] L. Gorvel, J. Textoris, R. Banchereau, A. Ben Amara, W. Tantibhedhyangkul, K. von Bargen, M.B. Ka, C. Capo, E. Ghigo, J.-P. Gorvel, J.-L. Mege, Intracellular bacteria interfere with dendritic cell functions: role of the type I interferon pathway, PloS One. 9 (2014) e99420. doi:10.1371/journal.pone.0099420.

[5] M.B. Ka, S. Mezouar, A. Ben Amara, D. Raoult, E. Ghigo, D. Olive, J.-L. Mege, Coxiella burnetii Induces Inflammatory Interferon-Like Signature in Plasmacytoid Dendritic Cells: A New Feature of Immune Response in Q Fever, Front. Cell. Infect. Microbiol. 6 (2016) 70. doi:10.3389/fcimb.2016.00070.

[6] J.S. Dahlin, J. Hallgren, Mast cell progenitors: Origin, development and migration to tissues, Mol. Immunol. 63 (2015) 9–17. doi:10.1016/j.molimm.2014.01.018.

[7] M. Urb, D.C. Sheppard, The Role of Mast Cells in the Defence against Pathogens, PLoS Pathog. 8 (2012). doi:10.1371/journal.ppat.1002619.

[8] I. Bot, T.J.. van Berkel, E.A.. Biessen, Mast Cells: Pivotal Players in Cardiovascular Diseases, Curr. Cardiol. Rev. 4 (2008) 170–178. doi:10.2174/157340308785160624.

[9] J.S. Dahlin, M. Ekoff, J. Grootens, L. Löf, R.-M. Amini, H. Hagberg, J.S. Ungerstedt, U. Olsson-Strömberg, G. Nilsson, KIT signaling is dispensable for human mast cell progenitor development, Blood. 130 (2017) 1785–1794. doi:10.1182/blood-2017-03-773374.

[10] R.M. Anthony, L.I. Rutitzky, J.F. Urban, M.J. Stadecker, W.C. Gause, Protective immune mechanisms in helminth infection, Nat. Rev. Immunol. 7 (2007) 975–987. doi:10.1038/nri2199.

[11] K. Amin, The role of mast cells in allergic inflammation, Respir. Med. 106 (2012) 9–14. doi:10.1016/j.rmed.2011.09.007.

[12] Y. Bechah, J. Verneau, A. Ben Amara, A.O. Barry, C. Lépolard, V. Achard, L. Panicot-Dubois, J. Textoris, C. Capo, E. Ghigo, J.-L. Mege, Persistence of Coxiella burnetii, the agent of Q fever, in murine adipose tissue, PloS One. 9 (2014) e97503. doi:10.1371/journal.pone.0097503.

[13] J. Cottalorda, J.L. Jouve, G. Bollini, P. Touzet, A. Poujol, F. Kelberine, D. Raoult, Osteoarticular Infection due to Coxiella Burnetii in Children, J. Pediatr. Orthop. B. 4 (1995) 219.

[14] A. Poujol, S. Toesca, J.M. Di, Y. Rimet, H.D. Tissot, L. Macé, N. Sudan, M. Panuel, Y.

Turcat, F. Kelberine, Y. Brusquet, [Recurrent osteitis and Coxiella burnetii: the relation to chronic recurrent multifocal osteomyelitis]., Arch. Pediatr. Organe Off. Soc. Francaise Pediatr. 5 (1998) 291–294. doi:10.1016/S0929-693X(97)89372-4.

[15] J.W. Whittick, Necropsy Findings in a Case of Q Fever in Britain, Br. Med. J. 1 (1950) 979–976.1.

[16] J.S. Li, D.J. Sexton, N. Mick, R. Nettles, V.G. Fowler, T. Ryan, T. Bashore, G.R. Corey, Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 30 (2000) 633–638. doi:10.1086/313753.

[17] J.-S. Shin, A.M. Greer, The role of FcεRI expressed in dendritic cells and monocytes, Cell. Mol. Life Sci. CMLS. 72 (2015) 2349–2360. doi:10.1007/s00018-015-1870-x.

[18] L. Escribano, M. Ocqueteau, J. Almeida, A. Orfao, J.F. San Miguel, Expression of the c-kit (CD117) molecule in normal and malignant hematopoiesis, Leuk. Lymphoma. 30 (1998) 459–466. doi:10.3109/10428199809057558.

[19] J.S. Dahlin, R. Feinstein, Y. Cui, B. Heyman, J. Hallgren, CD11c+ cells are required for antigen-induced increase of mast cells in the lung, J. Immunol. Baltim. Md 1950. 189 (2012) 3869–3877. doi:10.4049/jimmunol.1201200.

[20] D.P. Bailey, M. Kashyap, L.A. Bouton, P.J. Murray, J.J. Ryan, Interleukin-10 induces apoptosis in developing mast cells and macrophages, J. Leukoc. Biol. 80 (2006) 581–589. doi:10.1189/jlb.0405201.

[21] S. Meghari, Y. Bechah, C. Capo, H. Lepidi, D. Raoult, P.J. Murray, J.-L. Mege, Persistent Coxiella burnetii infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis, PLoS Pathog. 4 (2008) e23. doi:10.1371/journal.ppat.0040023.

[22] F. Ma, N. Kambe, D. Wang, G. Shinoda, H. Fujino, K. Umeda, A. Fujisawa, L. Ma, H. Suemori, N. Nakatsuji, Y. Miyachi, R. Torii, K. Tsuji, T. Heike, T. Nakahata, Direct development of functionally mature tryptase/chymase double-positive connective tissue-type mast cells from primate embryonic stem cells, Stem Cells Dayt. Ohio. 26 (2008) 706–714. doi:10.1634/stemcells.2007-0348.

[23] E.A. Pastorello, L. Farioli, L.M. Losappio, N. Morici, M. Di Biase, M. Nichelatti, J.W. Schroeder, L. Balossi, S. Klugmann, Serum tryptase detected during acute coronary syndrome is significantly related to the development of major adverse cardiovascular events after 2 years, Clin. Mol. Allergy CMA. 13 (2015) 14. doi:10.1186/s12948-015-0013-0.

~~[24] K.J. Filipiak, B. Tarchalska-Krynska, G. Opolski, A. Rdzanek, J. Kochman, D.A. Kosior, A. Czlonkowski, Tryptase levels in patients after acute coronary syndromes: the potential new marker of an unstable plaque?, Clin. Cardiol. 26 (2003) 366–372.~~

~~[25] G. P. Shi, I. Bot, P.T. Kovanen, Mast cells in human and experimental cardiometabolic diseases, Nat. Rev. Cardiol. 12 (2015) 643–658. doi:10.1038/nrcardio.2015.117.~~

Figure legend

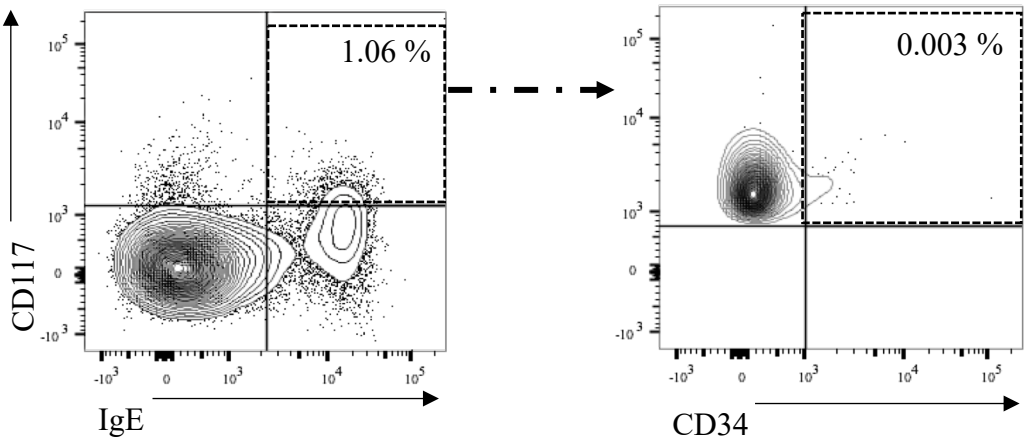
Figure 1. Mast cell progenitors in Q fever disease

PBMCs were recovered and analyzed by flow cytometry for the presence of MC progenitors using CD34, CD117 and IgE fluorescent markers. **(A)** Representative graph of percentage of cells expressing CD117, IgE and CD34. **(B)** The percentage of MC progenitors in PBMCs from healthy donors and Q fever **(C)** acute or endocarditis patients is shown. The nonparametric Mann-Whitney *U* test was used to compare control and patient groups. Horizontal bar, median value.

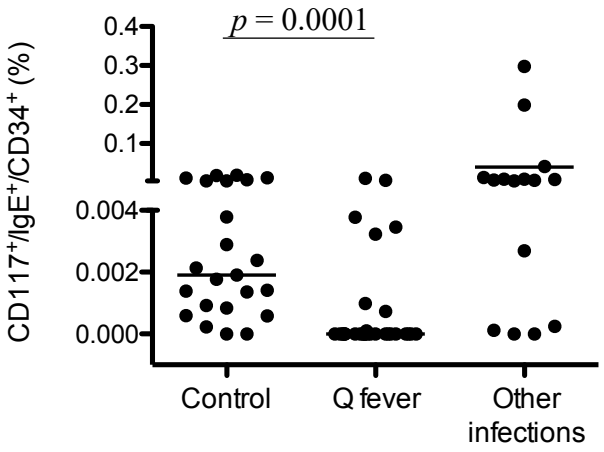
Figure 2. Serum basal tryptase in Q fever disease

(A) The serum basal tryptase of Q fever patients was assessed in comparison to control and other infections groups.

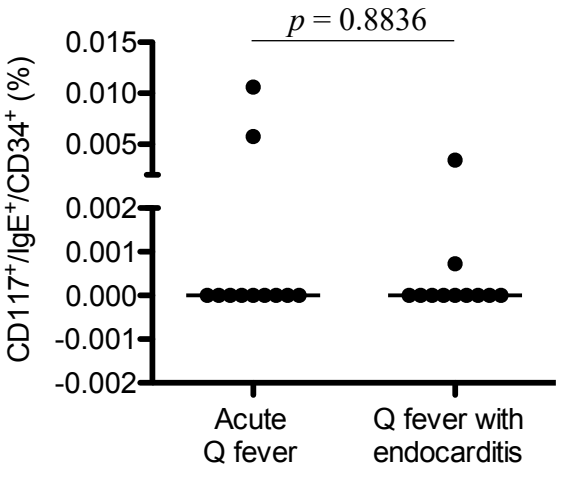
A.


1.06 %
0.003 %
CD117
IgE
CD34

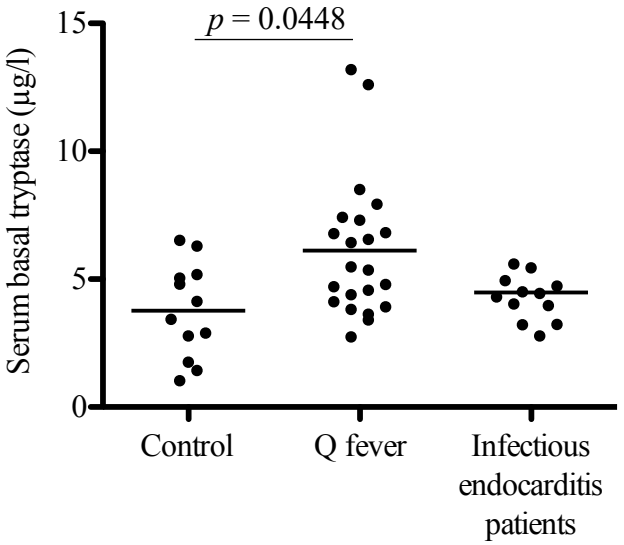
B.


p = 0.0001
CD117+/IgE+/CD34+ (%)
0.4
0.3
0.2
0.1
0.004
0.002
0.000
Control
Q fever
Other infections

C.


p = 0.8836
CD117+/IgE+/CD34+ (%)
0.015
0.010
0.005
0.002
0.001
0.000
-0.001
-0.002
Acute Q fever
Q fever with endocarditis

A.


p = 0.0448
15
10
5
0
Serum basal tryptase (µg/l)
Control
Q fever
Infectious endocarditis patients