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BALF CD103⁺CD4⁺/CD4⁺ ratio alone is enough to support the diagnosis of sarcoidosis in an appropriate clinicopathologic setting

We read with great interest the article by Mota et al. on the diagnostic value of CD103 expression in bronchoalveolar lymphocytes in sarcoidosis [1]. The authors conclude that a cutoff of 0.45 for the bronchoalveolar lavage fluid (BALF) CD103⁺CD4⁺/CD4⁺ ratio was associated with a better diagnostic performance for pulmonary sarcoidosis. Combined use of the BALF CD103/CD4 ratio <0.31 and the BALF CD4/CD8 ratio >2.5 was reported by Kolopp-Sarda et al. [2] and BALF CD103⁺CD4⁺/CD4⁺ ratio <0.2 and the BALF CD4⁺/CD8⁺ ratio >3 or BALF CD4⁺/CD8⁺ to peripheral blood (PB) CD4⁺/CD8⁺ ratio >2 has been reported by Heron et al. in the recent past as a specific tool for discriminating pulmonary sarcoidosis from other interstitial lung diseases (ILDs) [3]. As previously described in the literature [3–5], a higher proportion of CD103⁺CD4⁺ lymphocytes was found in advanced radiologic stages of pulmonary sarcoidosis in Mota et al.’s study [1]. A higher proportion of CD103⁺CD4⁺ lymphocytes is considered to be associated with development of fibrosis in pulmonary sarcoidosis [3–5].

The purpose of our study was to compare the flow cytometric ratios reported by Heron et al. [3] (i.e. the BALF CD103⁺CD4⁺/CD4⁺ ratio <0.2, the BALF CD4⁺ to CD8⁺ ratio >3 and the ratio of BALF CD4⁺ to CD8⁺ to PB CD4⁺ to CD8⁺ >2) with the biopsy proven sarcoidosis diagnosis. We received transbronchial and/or mediastinal biopsy specimens for morphologic examination and BALF for flow cytometric analysis (FCA) from 11 cases of ILDs with clinical and radiological suspicion of pulmonary sarcoidosis over a period of about 3 years. We also received peripheral blood drawn at the time of bronchoalveolar lavage procedure in 8 cases. Peripheral blood was not received from 3 patients. We performed FCA of BALF in all cases and of peripheral blood of 8 cases by using CD45, CD3, CD19, CD4, CD8, and CD103 antibodies (Becton Dickinson antibodies) on FACS Canto™ II system (Becton Dickinson) and correlated ratios reported by Heron et al. [3] with morphologic findings in the transbronchial and/or mediastinal biopsy specimens and also with Scadding’s radiologic stage (stage I: bilateral lymphadenopathy; stage II: bilateral lymphadenopathy combined with parenchymal involvement; stage III: exclusively parenchymal involvement; stage IV: fibrosis).

Our findings are presented in Table 1. Five out of 11 cases (cases 3, 4, 7, 9, and 10) had transbronchial and/or mediastinal biopsy proven sarcoidosis. Four of these five cases (cases 3, 7, 9, and 10) had stage I-II sarcoidosis and <0.2 BALF CD103⁺CD4⁺/CD4⁺ ratio. Only 2 of these 4 cases (cases 3 and 7) also met the other two ratios of Heron et al. [3] Fifth case (case 4) had stage IV (fibrotic) sarcoidosis but the BALF CD103⁺CD4⁺/CD4⁺ ratio was 0.26 (i.e. >0.2) and did not meet the other two ratios of Heron et al. [3] Six of 11 cases (cases 1, 2, 5, 6, 8, and 11) that did not have sarcoidosis were treated as other forms of ILDs. These 6 cases were the only controls used in this small study. Diagnosis of sarcoidosis was established or ruled-out by correlating FCA findings and transbronchial and/or mediastinal biopsy findings. Because of the very small size of our study, we did not use any statistical methods to calculate sensitivity and specificity. One of 6 non-sarcoidosis cases (case 5) had BALF CD4⁺/CD8⁺ ratio >3 as well as BALF CD4⁺/CD8⁺ to PB CD4⁺/CD8⁺ ratio >2 and another case (case 11) had BALF CD4⁺/CD8⁺ ratio >3, but only 2 of these 6 cases (cases 1 and 6) that did not have alveolar lymphocytosis (i.e. <10% lymphocytes as defined in the literature) had less than 0.2 ratio of BALF CD103⁺CD4⁺ to CD4⁺. These two cases (cases 1 and 6) did not show non-caseating granulomas in the biopsy specimens and, therefore, did not have sarcoidosis. Although BAL lymphocytosis is not a universal finding in sarcoidosis [6], we did not get any help from FCA by using the criterion of <0.2 ratio of BALF CD103⁺CD4⁺ to CD4⁺ in the diagnosis of sarcoidosis if there was no alveolar lymphocytosis (i.e. at least 10% lymphocytes). Heron et al. [3], Kolopp-Sarda et al. [2] and Mota et al. [1] included only patients with alveolar lymphocytosis and it appears that the presence of alveolar lymphocytosis should be a pre-requisite for the application of the aforementioned flow cytometric criteria for the diagnosis of pulmonary sarcoidosis. To emphasize the requirement of the presence of alveolar lymphocytosis for performing FCA of the BALF in the differential diagnosis of pulmonary sarcoidosis and other ILDs, we can look at the conclusion drawn by Hyldgaard et al. [7] in their study published a few months earlier than the publication by Mota et al. [1]. Hyldgaard et al. [7] did not find any significant differences in BALF CD103⁺/CD4⁺/CD4⁺ ratio between sarcoidosis patients and the patients with other ILDs. The combinations of CD103⁺/CD4+/CD4⁺, CD4⁺/CD8⁺ and lymphocyte percentages that were tested in their study demonstrated only a limited diagnostic value in their unselected group of sarcoidosis patients. The criteria proposed by Heron et al. [3] and Kolopp-Sarda et al. [2] based on combinations of CD103⁺/CD4+/CD4⁺ and CD4⁺/CD8⁺ had a lower sensitivity in Hyldgaard et al. [7] study than those reported by Heron et al. [3] and Kolopp-Sarda et al. [2]. They thought that one of the explanations for their lower sensitivity than that reported by the previous studies was that their patients were not pre-selected for alveolar lymphocytosis. Therefore, the FCA of BALF should be performed
in the work-up of ILDs only if there is alveolar lymphcytosis.

The conclusions of our small study are:

1. BALF CD103+/CD4+ ratio <0.2 can be used as the sole flow cytometric criterion for the diagnosis of non-fibrotic sarcoidosis in the correct clinicopathologic setting, but only if at least 10% lymphocytes are available in the BALF for FCA. We define correct clinicopathologic setting for sarcoidosis based on three criteria mentioned by Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) and adopted by the ATS Board of Directors and by the ERS Executive Committee in February 1999. The three criteria are: 1) a compatible clinical and/or radiological presentation; 2) histological evidence of noncaseating granulomas; and 3) exclusion of other diseases that produce a similar histological or clinical picture [8]. At this time, we do not recommend using BALF CD103+/CD4+ ratio <0.2 as a sole criterion for the diagnosis of pulmonary sarcoidosis, but as a sole criterion in the correct clinicopathologic setting and actually recommend adding it as a fourth criterion to the aforementioned 3 criteria.

2. High BALF CD4+/CD8+ ratio >3 as well as high ratio or BALF CD4+/CD8+ to PB CD4+/CD8+ ratio >2 can be seen in non-sarcoidosis ILDs. Therefore, these criteria are not reliable in differentiating pulmonary sarcoidosis from other ILDs.

3. BALF CD103+/CD4+ ratio <0.2 can be seen in non-sarcoidosis ILDs if the number of lymphocytes available for FCA in the BALF are too low (i.e., <10%). Therefore, we agree with Heron et al. [3], Kolopp-Sarda et al. [2] and Mota et al. [1] that the presence of alveolar lymphcytosis is a pre-requisite for the application of the aforementioned flow cytometric criteria for the diagnosis of pulmonary sarcoidosis.

As our study included only a small number of cases, we had suggested a larger study to confirm our conclusion that BALF CD103+/CD4+ ratio <0.2 alone is enough for the diagnosis of non-fibrotic (Scadding’s radiologic stage 0-III) sarcoidosis in an appropriate clinicopathologic setting. We had presented our findings in a poster at the 2011 annual College of American Pathologists’ meeting and our findings were also published last year as an abstract [9].

Since we published the findings of our small study as an abstract, we have done FCA on additional 4 patients with clinical/radiologic suspicion of sarcoidosis. The results of these 4 patients are shown in Table 2. One of these 4 patients (case 1) had bilateral pulmonary reticular densities with fibrosis (Scadding’s stage IV), a BALF CD103+/CD4+ ratio 0.448, a BALF CD4+/CD8+ ratio 11.1, and a BALF CD4+/CD8+ to PB CD4+/CD8+ ratio 10.1. Therefore, this case met Mota et al.’s criterion of a BALF CD103+/CD4+ ratio <0.45 and two other criteria mentioned in the literature [2,3]. Two of other 3 patients (cases...
2 and 3) had bilateral pulmonary reticular densities and bilhar lymphadenopathy (Scadding’s stage II). One of these 2 patients (case 2) showed a BALF CD103+/CD4+ ratio 0.06, a BALF CD4+/CD8+ ratio 10.9 and BALF CD4+/CD8+ to peripheral PB CD4+/CD8+ ratio 7.3. This patient met all flow cytometric criteria described so far for the diagnosis of sarcoidosis [2,3]. Other patient (case 3) demonstrated a BALF CD103+/CD4+/CD8+ ratio 0.13, a BALF CD4+/CD8+ ratio 3.3 and a BALF CD4+/CD8+ to PB CD4+/CD8+ ratio 1.8. This patient fulfilled all three BALF CD103+/CD4+ to CD4+ ratio criteria described so far (<0.2, <0.31 and < 0.45) and the criterion of >3.0 BALF CD4+/CD8+ ratio [1–3]. Fourth patient had bilateral pulmonary reticular nodules (Scadding’s stage III) and showed a BALF CD103+/CD4+/CD8+ ratio 0.10, a BALF CD4+/CD8+ ratio 1.1 and BALF CD4+/CD8+ to peripheral PB CD4+/CD8+ ratio 1.4. This patient met only BALF CD103+/CD4+/CD8+ ratio flow cytometric criterion described in the literature [1–3].

Based on a small number of cases we have studied, we think that a <0.2 ratio of CD103 expressing CD4+ cells to the total number of CD4+ cells in the BALF is a very reliable criterion for the diagnosis of non-fibrotic sarcoidosis in patients with a strong clinicopathologic suspicion of sarcoidosis. We agree that a ratio <0.2 is too low to be helpful in the diagnosis of Scadding’s stage IV (fibrotic) sarcoidosis and a higher ratio can help diagnose fibrotic stage of sarcoidosis. Two abovementioned patients who had sarcoidosis with fibrosis had this ratio at >0.20 and at <0.45 (case 4 in Table 1 and case 1 in Table 2). Heron et al. [3], who described this ratio at <0.2, did see higher than >0.2 ratio in higher radiologic stage sarcoidosis in their study. Mota et al. [1] also mention a ratio higher than 0.2 for higher radiologic stages of sarcoidosis and have refined the ratio to <0.45 for better diagnostic performance for sarcoidosis. It appears that the refined ratio of 0.45 described by them is a good cutoff point that will cover the diagnosis of all radiologic stages of pulmonary sarcoidosis in the correct clinicopathologic setting.

Mota et al. [1] have done the large study that we had wished to be done to confirm or refute our conclusion that BALF CD103+/CD4+/CD8+ ratio <0.2 is enough as a criterion, irrespective of the BALF CD4+/CD8+ ratio or BALF CD4+/CD8+ to PB CD4+/CD8+ ratio, for the diagnosis of non-fibrotic (Scadding’s radiologic stage IV) sarcoidosis. Actually their study helps more than what we had expected by concluding that the BALF CD103+/CD4+/CD8+ ratio <0.45 can be used alone (without looking at BALF CD4+/CD8+ ratio or BALF CD4+/CD8+ to PB CD4+/CD8+ ratio) for supporting the diagnosis of pulmonary sarcoidosis irrespective of the radiologic stage in an appropriate clinicopathologic setting.

Conflict of interest statement

The authors declare no conflict of interest.

References


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