ORIGINAL RESEARCH LETTER

Detection of *Cutibacterium acnes* in granulomas of patients with either hypersensitivity pneumonitis or vasculitis reveals that its presence is not unique for sarcoidosis

To the Editor:

Granulomas are compact organised structures of different immune cells, including macrophages, lymphocytes and plasma cells, thought to be formed when (foreign) antigens cannot be cleared. The differential diagnosis of a granulomatous lesion is broad and includes infectious aetiologies, malignancy and inflammatory disorders like vasculitis, hypersensitivity pneumonitis and sarcoidosis [1].

Cutibacterium acnes, formerly *Propionibacterium acnes*, is a Gram-positive bacterium that is a commensal of the skin [2]. Studies have demonstrated that *C. acnes* is also able to induce granulomas [3]. Furthermore, *C. acnes* is suggested to be involved in the pathogenesis of sarcoidosis, a complex inflammatory disease mainly involving the lungs and lymph nodes, characterised by noncaseating granulomas [4].

Although presence of *C. acnes* has already been shown in granulomas of sarcoidosis patients [5], it is unknown whether this bacterium can also be found in granulomatous disorders with frequent involvement of the lungs other than sarcoidosis, such as hypersensitivity pneumonitis (HP), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA).

To investigate whether the presence of *C. acnes* in granulomas is specific to sarcoidosis, we examined granulomatous tissue of HP, GPA and EGPA patients for the presence of *C. acnes*.

Tissue blocks were collected from patients with HP, GPA or EGPA who participated in our biobank study. Patients were included in the study when enough residual tissue was available and when granulomas could be detected in the haematoxylin-stained tissue sections. Tissue blocks of 35 patients with HP and of 13 patients with (E)GPA were collected. Tissue blocks of all HP and nine (E)GPA patients showed granulomas and had enough tissue left to be included in the study. The study was approved by the Medical Research Ethics Committees United of the St Antonius Hospital (R05-08A) and written consent was obtained from all patients. Formalin-fixed, paraffin-embedded tissue sections were immunohistochemically stained with the PAB antibody, a *C. acnes*-specific monoclonal antibody that binds to the cell membrane-bound lipoteichoic acid (LTA) of the bacterium [5]. Full methods are described elsewhere [6]. Differences in the presence of *C. acnes* between the patient groups was compared using the Chi-squared test. An independent-sample t-test was used to compare bronchoalveolar lavage (BAL) lymphocytes between HP patients. Fisher's exact test was used to compare presence of *C. acnes* in granulomas of HP patients with and without a known inducing agent. p-values <0.05 were considered significant.

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Presence of *C. acnes* in granulomas is not unique to sarcoidosis but can also be found in patients with HP or EGPA. *C. acnes* may be involved in the pathogenesis of those granulomatous diseases in a mitogenic way. https://bit.ly/3pU0PeC

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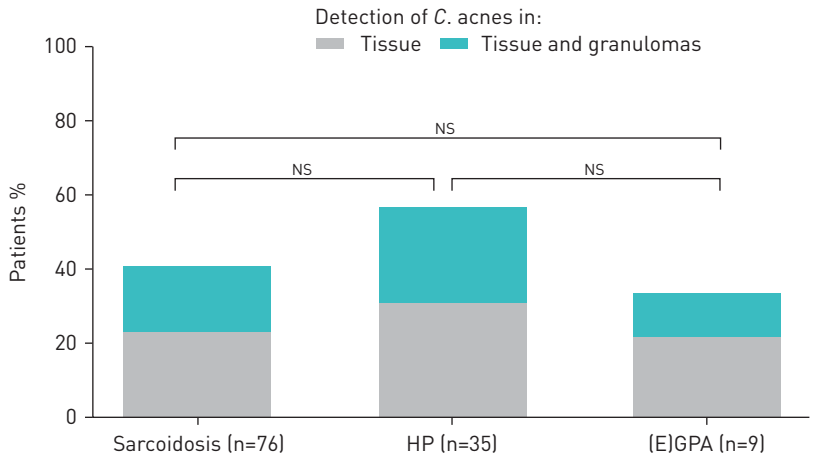
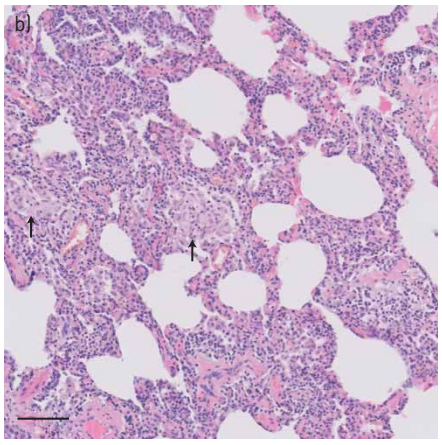
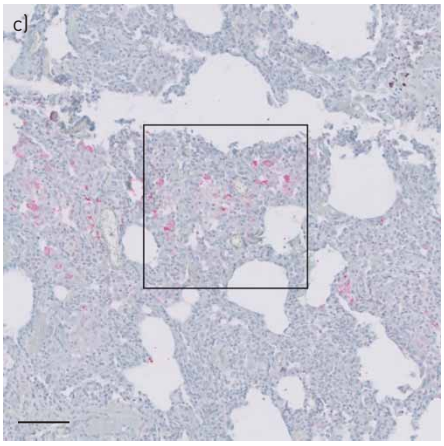
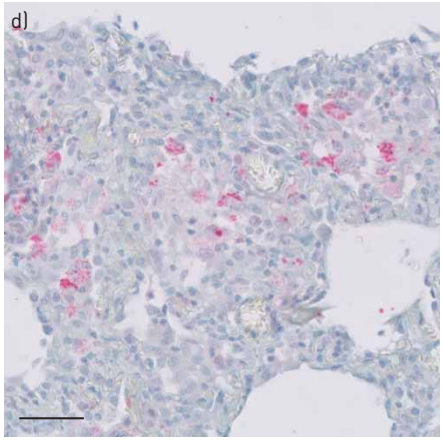
a)

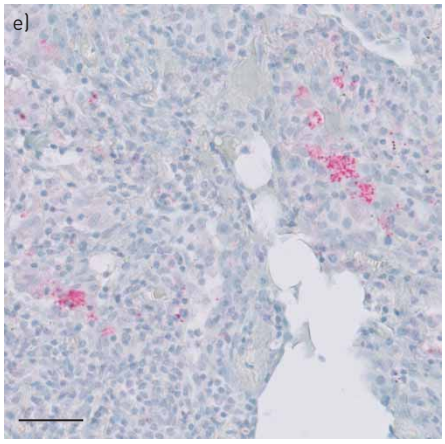


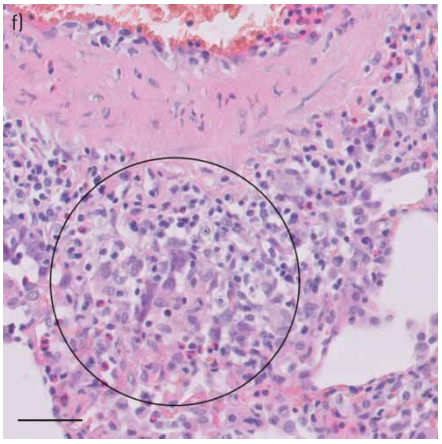
Age years mean ±SD	44.0±12.2	58.2±9.2	51.6±14.7
Male sex	42 (55.3)	17 (48.6)	6 (66.7)
Origin of tissue			
Lung	30 (39.5)	34 (97.1)	6 (66.7)
Lymph node	24 (31.6)	1 (2.9)	
Skin	17 (22.4)		1 (11.1)
Nose	1 (1.3)		2 (22.2)
Other	4 (5.3)		
Inducing agent			
Birds	NA	14 (40.0)	NA
Farmer's lung		3 (8.6)	
Unknown		18 (51.4)	

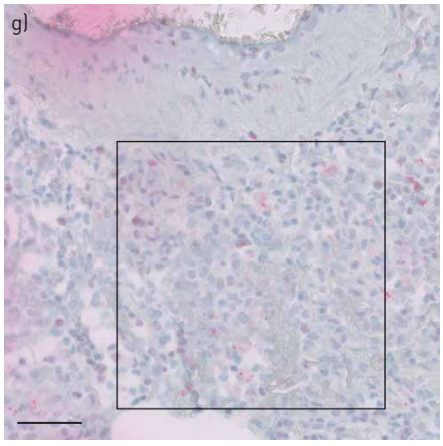


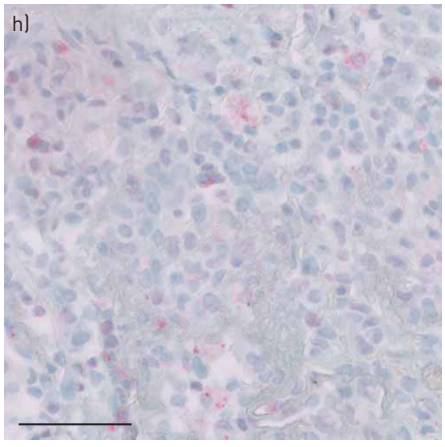












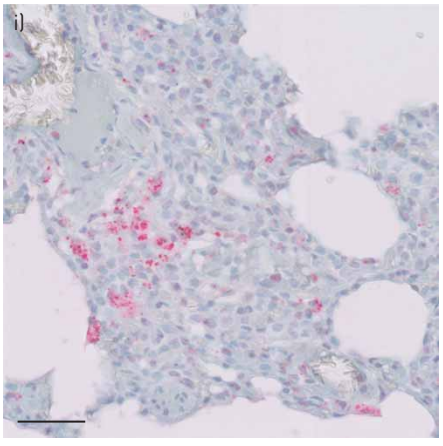


FIGURE 1 Presence of *Cutibacterium acnes* in tissue and granulomas of patients with hypersensitivity pneumonitis (HP) and (eosinophilic) granulomatosis with polyangiitis ((E)GPA). a) Characteristics of all included patients and percentage of patients with presence of *C. acnes* in tissue or in tissue and granulomas. The sarcoidosis group was previously described [6]. No significant difference was observed between the three groups regarding *C. acnes* in tissue or *C. acnes* in granulomas (p=0.210 and p=0.460, respectively). Data are presented as n (%) unless otherwise stated. b) Haematoxylin and eosin stain (HE) stain of lung tissue from an HP patient. The black arrows show granulomas. Scale bar=100 µm. c) Positive PAB immunohistochemistry (red) in the granulomas corresponding to (b). Scale bar=100 µm. d) Higher-magnification image corresponding to the square in picture (c). Scale bar=50 µm. e) Area of positive PAB immunohistochemistry outside granulomas. Scale bar=50 µm. f) HE stain of lung tissue from an EGPA patient, with an immature granuloma indicated by the circle. Scale bar=50 µm. g) Positive PAB immunohistochemistry in the granuloma corresponding to (f). Scale bar=50 µm. h) Higher-magnification image corresponding to the square in (g). Scale bar=50 µm. i) Area of positive PAB immunohistochemistry outside granulomas. Scale bar=50 µm. NS: nonsignificant; NA: not applicable.

Presence of *C. acnes* in tissue was found in 57.1% of HP patients (20 out of 35) and in 33.3% of (E)GPA patients (three out of nine). More specifically, the presence of *C. acnes* was observed inside granulomas of 25.7% HP patients (nine out of 35) and of 11.1% (E)GPA patients (one out of nine). Results between the diagnostic groups were not statistically significant (p=0.272 and p=0.659 for presence of *C. acnes* in tissue and granulomas, respectively). Moreover, the percentages of patients with presence of *C. acnes* in tissue or granulomas was comparable with a previously described Dutch sarcoidosis cohort (figure 1). As the presence of *C. acnes* appeared not to be specific to sarcoidosis and considering the known attributed mitogenic properties of this bacterium [7], we further explored such a role for *C. acnes* in our study by assessing BAL lymphocytosis in the patients with HP. Interestingly, a higher percentage of lymphocytes was indeed observed in HP patients that stained positively for *C. acnes* in granulomas compared to HP patients without *C. acnes* staining in granulomas (70.3% *versus* 41.3%, p=0.018). The percentage of patients with presence of *C. acnes* in granulomas did not significantly differ between the HP group in which an inducing agent was known compared to the HP group in which the inducing agent was unknown (35.3% *versus* 16.7%, p=0.264).

Up to now, considering granulomatous diseases, *C. acnes* has solely been related to the pathogenesis of sarcoidosis [8]. Presence of *C. acnes* has been demonstrated in tissue and granulomas of Japanese, German and Dutch sarcoidosis patients [5, 6]. To the best of our knowledge, this is the first study that demonstrates that *C. acnes* can also be detected in tissue and granulomas of patients with HP and vasculitis. If the presence of *C. acnes* is not disease specific, as our data suggest, one can debate whether *C. acnes* has an antigenic role in sarcoidosis pathogenesis. Regarding HP and (E)GPA pathogenesis, no data are available suggesting an antigenic role for *C. acnes*. However, data on a possible mitogenic role for *C. acnes* have been described [7, 9]. The higher percentage of lymphocytes observed in BAL of HP patients with presence of *C. acnes* in granulomas, supports the hypothesis that *C. acnes* may indeed act as a mitogen by enhancing lymphocyte proliferation. Moreover, since we observed presence of *C. acnes* in granulomas of HP patients in which a causal agent was previously identified, it is unlikely that *C. acnes* act as a specific agent in HP.

The PAB antibody that was used in this study reacts with the cell membrane-bound LTA of the *C. acnes* bacteria. LTA is a cell wall polymer of Gram-positive bacteria and plays a role in bacterial growth, membrane homeostasis and virulence [10]. Furthermore, LTAs have shown to be immunogenic [11] and activate the innate immune system *via* toll-like receptor 2 (TLR2) and NOD-like receptor family pyrin domain-containing 6 (NLRP6) [12, 13], key receptor families involved in the innate immune defence against invading pathogens. Interleukin-18 production can be stimulated following activation of NLRP6 [13]. Results of several studies suggest that TLR2 and interleukin-18 are at least partly involved in granuloma formation [14, 15], and *in vitro* and *in vivo* models have indeed shown that *C. acnes* is able to induce granulomas [3]. It is therefore possible that *C. acnes* is not a specific trigger of sarcoidosis, HP and (E)GPA, but that its LTA contributes to inflammation and granuloma formation.

A limitation of the study is that no control group with healthy individuals was included in the study. It is, however, difficult to include an appropriate control group in the study, as healthy controls would usually not show granulomas in tissue. Another limitation was that we have not been able to analyse the disease course in HP patients due to the small sample size in combination with too many missing follow-up data. Furthermore, the sample size of vasculitis patients from whom suitable tissue was available was too small to perform a subanalysis on disease course or BAL. Consequently, we were unable to examine whether *C. acnes* is related to a chronic disease course in (E)GPA and HP as well. Due to the low sample sizes of the HP and EGPA groups, the power to detect a difference with the sarcoidosis group was too small to conclude that those diseases, and HP patients with and without a known inducing agent, do not really differ regarding presence of *C. acnes* in granulomas. Although the percentages of *C. acnes* in granulomas of the nonsarcoidosis group were quite comparable to the sarcoidosis group, further studies using a higher number of patients are needed to clarify whether there is really no difference in presence of *C. acnes* between those diseases. Last, in this study we only examined LTA of the *C. acnes* bacteria. It is plausible

that LTAs of other Gram-positive bacteria can also be found in granulomas. Future studies will have to determine whether granulomas are uniquely related to LTAs of *C. acnes* or can be attributed to LTAs in general.

To conclude, we have shown that presence of *C. acnes* in granulomas is not unique for sarcoidosis but can also be found in patients with HP and EGPA. We hypothesise that *C. acnes* may be involved in the disease pathogenesis of those granulomatous diseases in a mitogenic way. Future studies are needed to determine the precise role of *C. acnes* and other LTAs in those granulomatous diseases.

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