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Early View

Research letter

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

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Conflicts of interest

The authors have nothing to disclose

Take home message

Presence of *C. acnes* in granulomas is not unique for sarcoidosis but can also be found in patients with HP and EGPA. *C. acnes* may be involved in the disease pathogenesis of those granulomatous diseases in a mitogenic way.

Granulomas are compact organized 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 (*C. acnes*) formerly *Propionibacterium acnes*, is a gram positive bacteria 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, characterized by non-caseating 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 presence of *C. acnes* in granulomas is specific for sarcoidosis we examined granulomatous tissue of HP, GPA and EGPA patients for presence of *C. acnes.*

Tissue blocks were collected from patients with HP, GPA and 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 9 (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 (MEC-U) 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 reacts with cell-membrane bound lipoteichoic acid (LTA) of the bacterium

(5). Full methods are described elsewhere (6). Difference in presence of *C. acnes* between the patients groups was compared using the Chi-squared test. An independent sample T-test was used to compare 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 an known inducing agent. P-values <0.05 were considered significant.

Presence of *C. acnes* in tissue was found in 57.1% of HP patients (20/35) and in 33.3% of (E)GPA patients (3/9). More specifically, presence of *C. acnes* was observed inside granulomas of 25.7% HP patients (9/35) and of 11.1% (E)GPA patients (1/9). 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 presence of *C. acnes* appeared not to be specific to sarcoidosis and considering the known attributed mitogenic properties of this bacteria (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 stained positively for *C. acnes* in granulomas compared to HP patients without *C. acnes* staining in granulomas (70.3% vs 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% vs 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 presence of *C. acnes* is not disease specific, as our data suggests, one can debate whether *C. acnes* has an antigenic role in sarcoidosis pathogenesis. Regarding HP and (E)GPA

pathogenesis no data is 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 an 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 has been used in this study reacts with cell-membrane bound lipoteichoic acid (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 papers 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 much missing follow-up data. Furthermore, also the sample size of vasculitis patients from whom suitable tissue was available was too small to perform sub analysis 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 samples sizes of the HP and EGPA group, 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 non-sarcoidosis 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 hypothesize 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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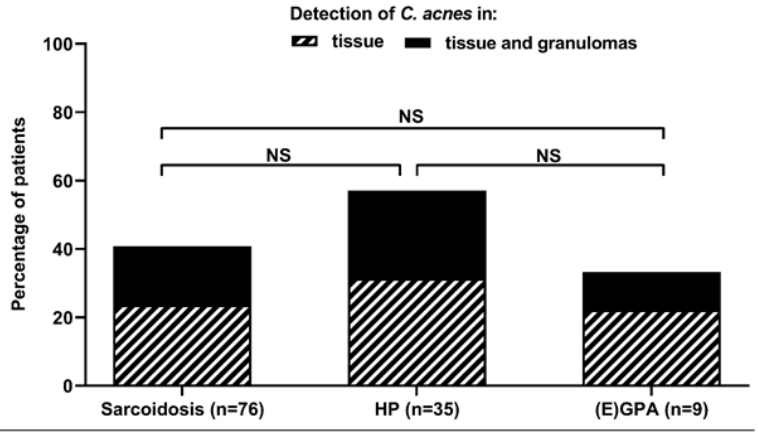
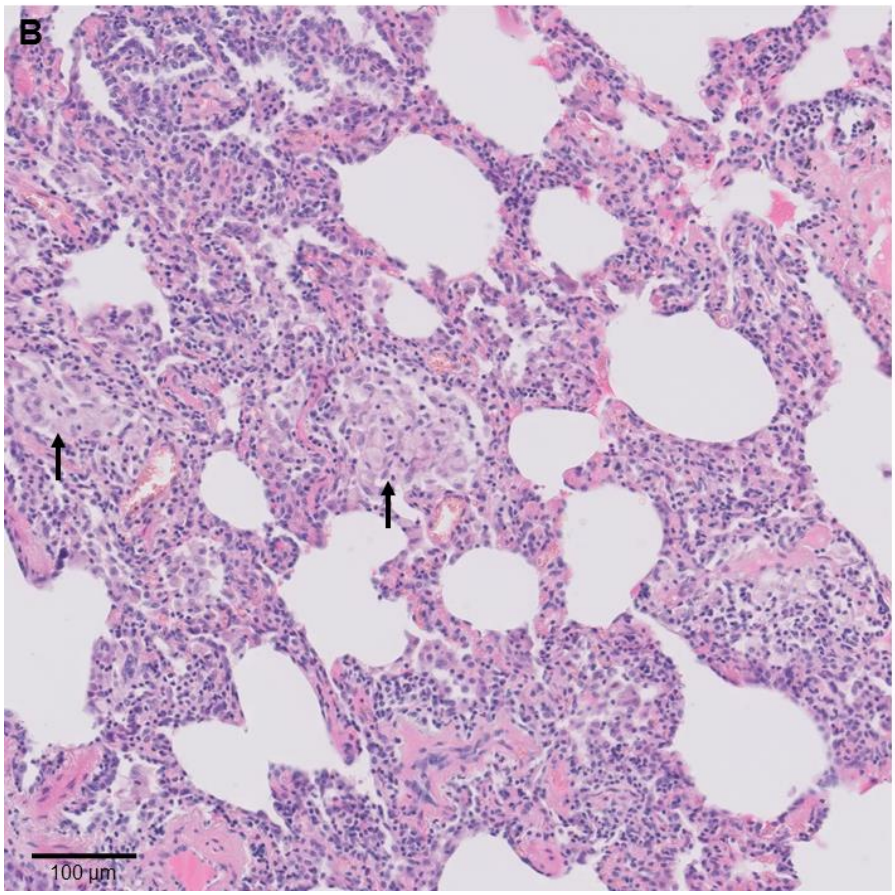
Contributions

EB contributed to data acquisition and analysis, statistical analysis and drafting and editing of the manuscript. KS participated in the design of the study, scored the staining results and edited the manuscript. BM edited the manuscript. JD cared out all the immunohistochemical stainings and developed the automated staining protocol. MQ contributed to data acquisition and edited the manuscript. JG supervised the project and edited the manuscript. MV designed the study, supervised the project and edited the manuscript.

A



Age (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)	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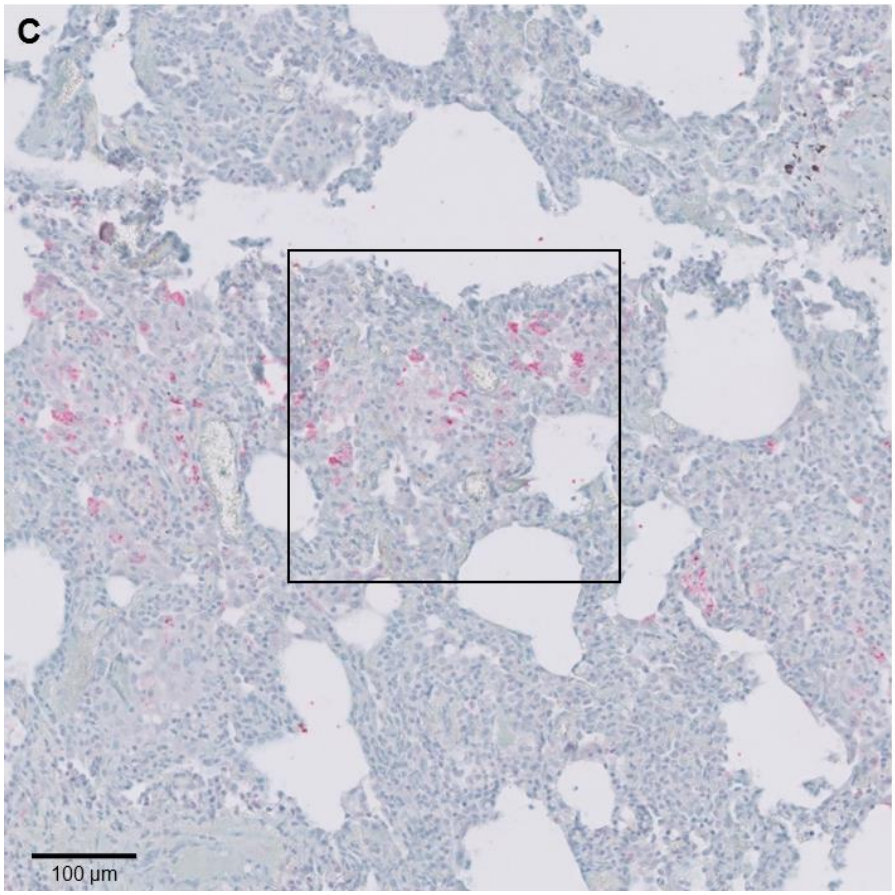
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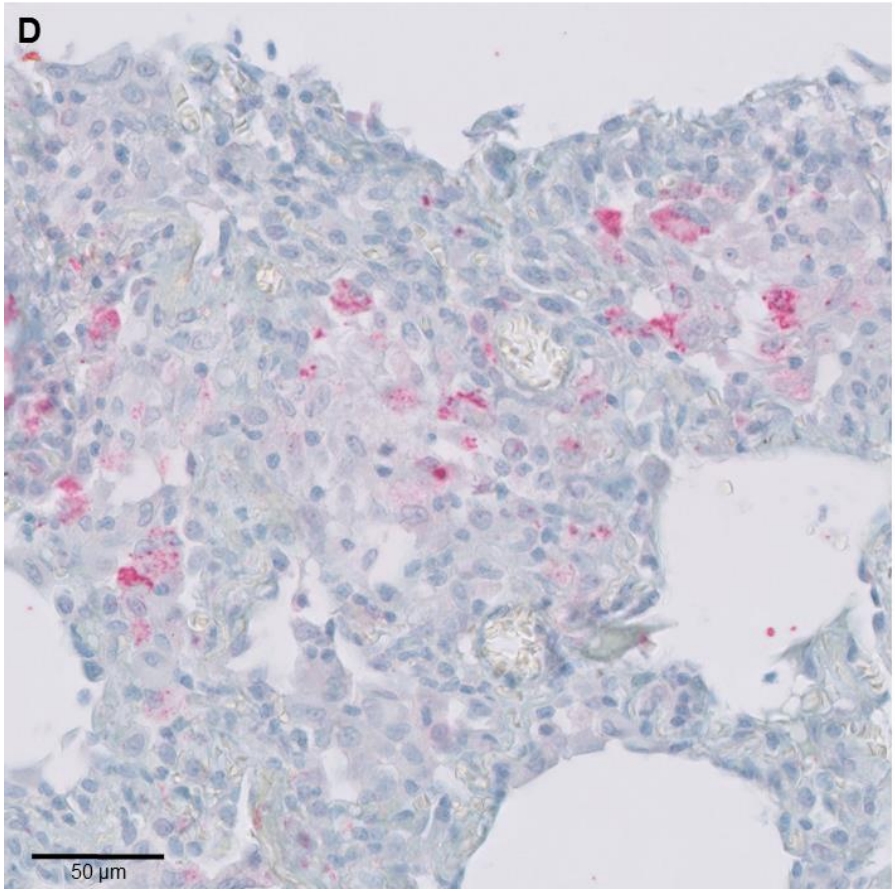
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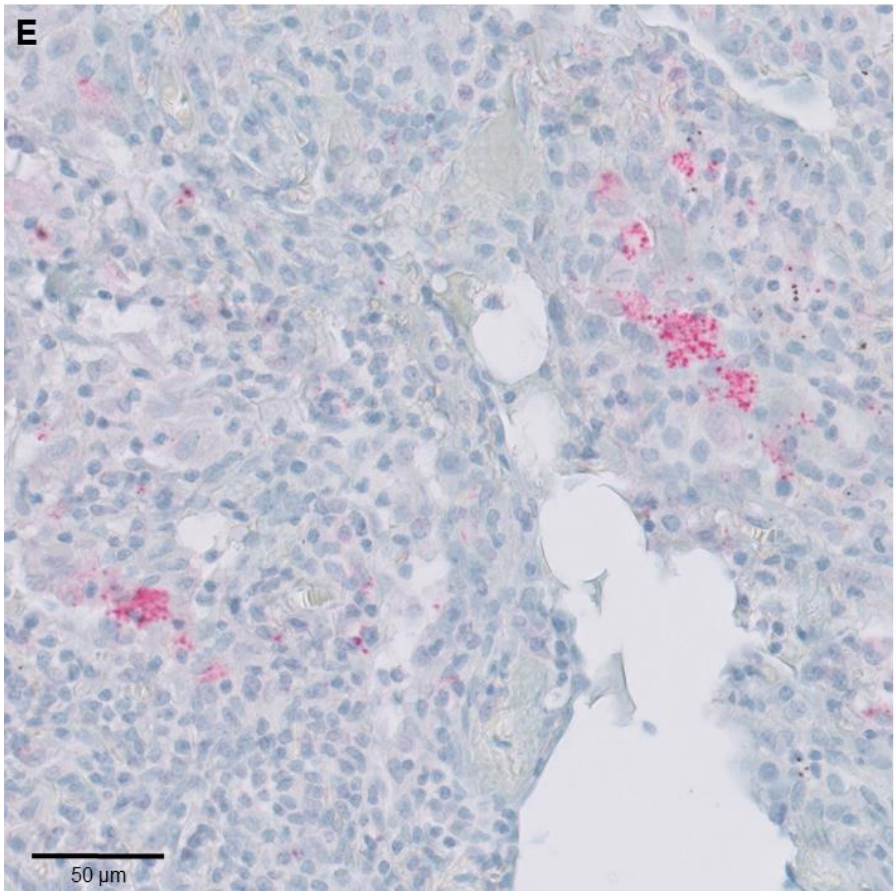
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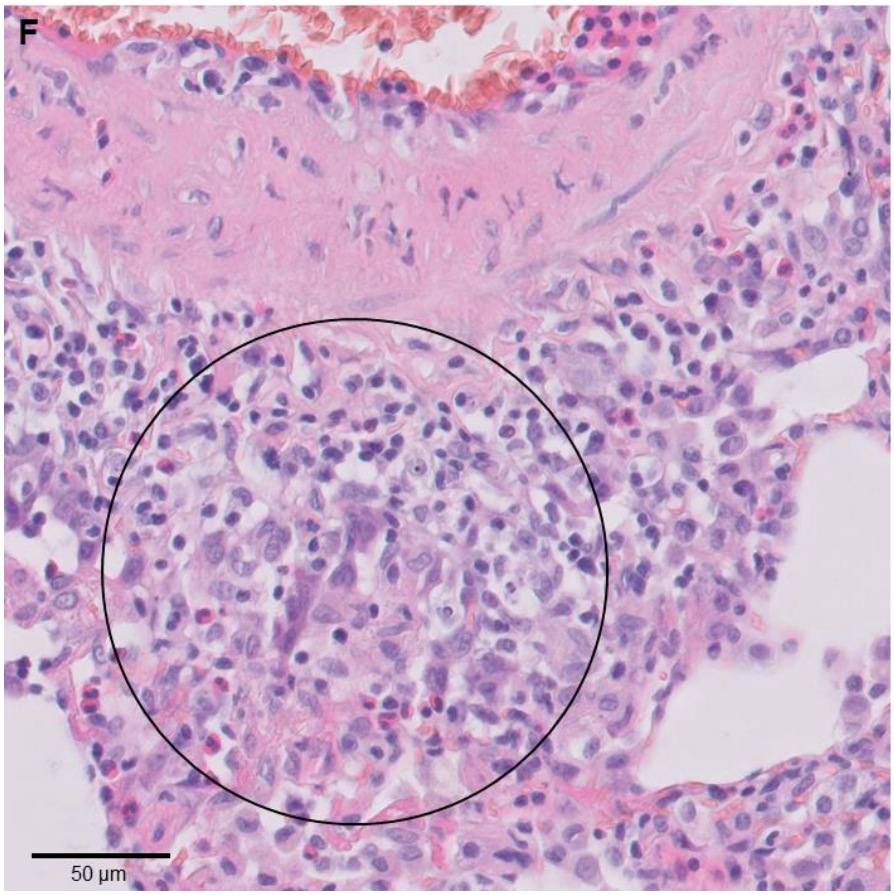
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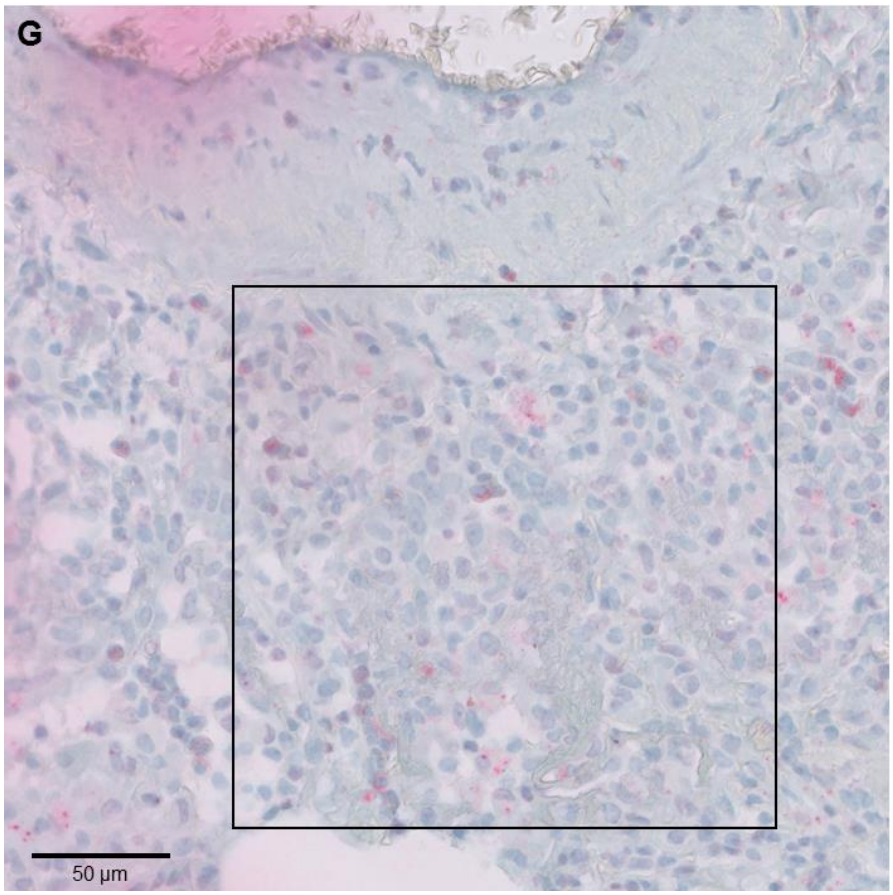
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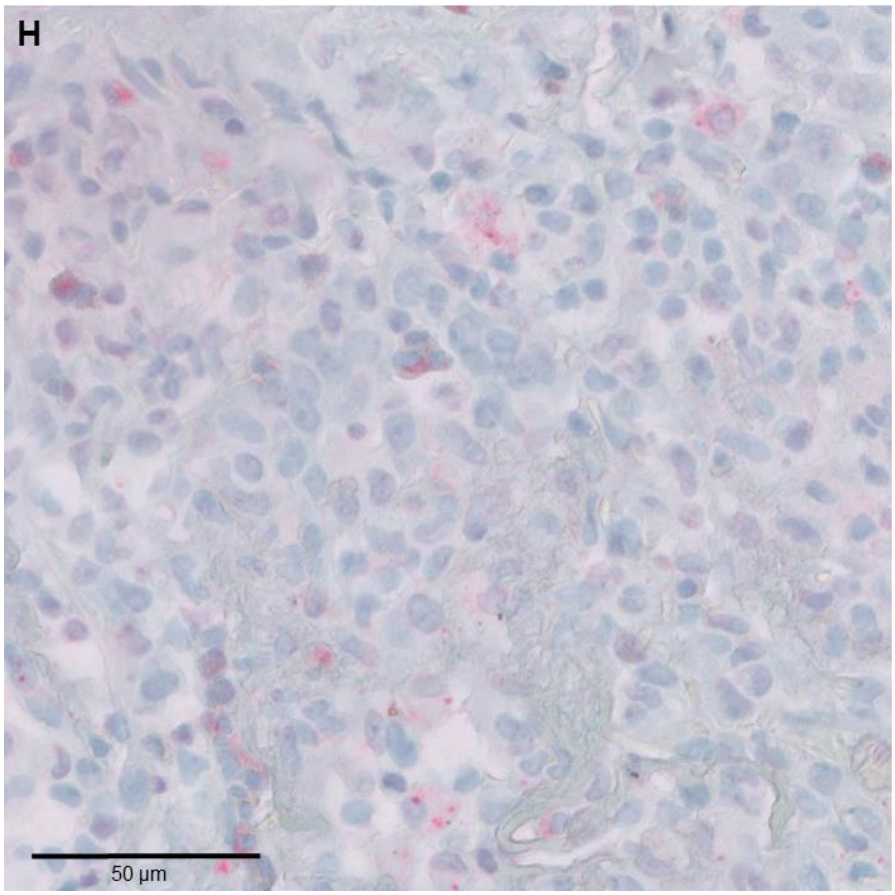
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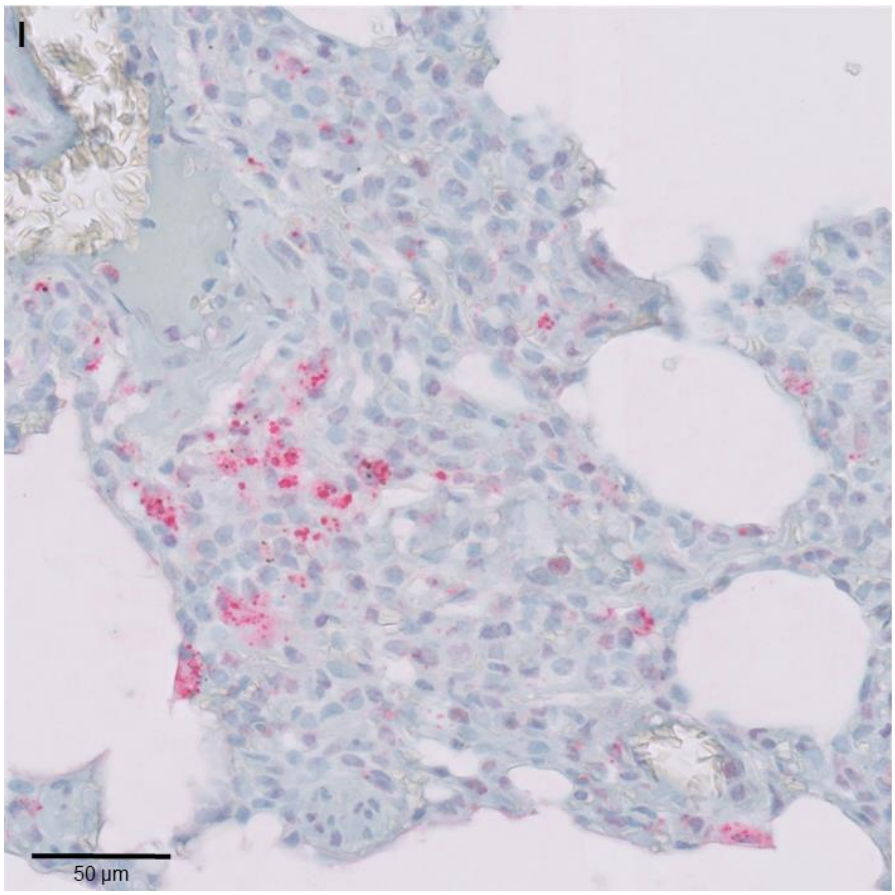

Figure 1. Presence of C. acnes in tissue and granulomas of HP (B to E) and (E)GPA (F to I) patients.
A. Characteristics of all included patients (below) and percentage of patients with presence of C. acnes in tissue or in tissue and granulomas (above). The sarcoidosis group was previously described (6). 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). B. Haematoxylin and eosin stain (HE) stain of lung tissue from a HP patient. The black arrows show granulomas. C. Positive PAB staining (in red) in the granulomas corresponding with picture B. D. Higher magnification corresponding with the square in picture C. E. Area of positive PAB staining outside granulomas.
F. HE stain of lung tissue from a EGPA patient, with an immature granuloma indicated by the circle. G. Positive PAB staining (in red) in the granuloma corresponding with picture G. H. Higher magnification corresponding with the square in picture H. I. Area of positive PAB staining outside granulomas.
HP: hypersensitivity pneumonitis, (E)GPA: (Eosinophilic) granulomatosis with polyangiitis, PAB: C. acnes specific monoclonal antibody that reacts with cell-membrane bound lipoteichoic acid (LTA) of the bacterium. NA: not applicable, NS: non-significant