Illustrations and Further Clarification for Radiomics Granuloma Classification System

Introduction

Subsequent to the development of a Histological Granuloma Classification System for the Radiomics Project, it seemed reasonable to provide illustrations of prototypical lesions for the various categories being used. But what is prototypical? Indeed, if even most of the granulomas we harvest and examine were standard, uniform and consistent/typical for a classification scheme, life would be much simpler. But think about what these lesions actually represent – a dynamic pathological process almost always changing per the influence of the ongoing host immune response. What we end up with in the glass slides generated is a snapshot of that lesion according to its status the day of harvest. We don't know what it looked like last month or what it might become next month – only that it was/will likely be different than today.

A lesion may develop initially as a tiny focus of granulomatous alveolitis at the site of deposition of an inhaled bacillus, expand to a larger, unorganized area of inflammation and eventually begin evolving the structural architecture and organization of a granuloma based on the influence of a myriad of different cell types, cytokine effects, etc. And if a granuloma does form, that structure is also not fixed or static, but a dynamic entity based on continued host immune balance. So non-necrotizing lesions can progress to necrotizing and then back, active foci can become contained and vice versa, lesions can wall off infection and begin early or even advanced healing in the form of fibrosis or mineralization only to reactivate and become uncontrolled once more, etc, etc. I realize that you all get this – but I restate it as we begin this endeavor only to emphasize that the development of a classification system for such a contiguous, ever changing process is inherently risky – necessary for the intent of the project, but fraught with potential flaws. For example, in order to have any significant value, such a system must possess a wide enough range of classification types to allow one to reasonably distinguish a variety of lesions and correlate them with other parameters. Things though are rarely black or white, but often shades of gray. But how many shades do we define? Too few choices and the scheme may have little predictive value. On the other hand, too many categories when dealing with the inherent subjectivity histomorphological assessment entails generally leads to confusion, a lack of repeatability and labor intensity to the point of impracticality. For example, we could score each granuloma from zero to three with regard to neutrophil composition, epithelioid macrophages, multinucleated giant cells, eosinophils, lymphoplasmacytic component, internal fibrous organization, peripheral fibrosis, degree of mineralization, etc, etc – but what would we match all that with and to what practical end?

That cautionary note aside, I think what has been put forth (4 main granuloma types with a total of 9 possible categorical classifications) is a logical starting point. Enough to recognize basic intrinsic compositional differences reflective of general disease state, but not detailed to the point of hopeless subjectivity.

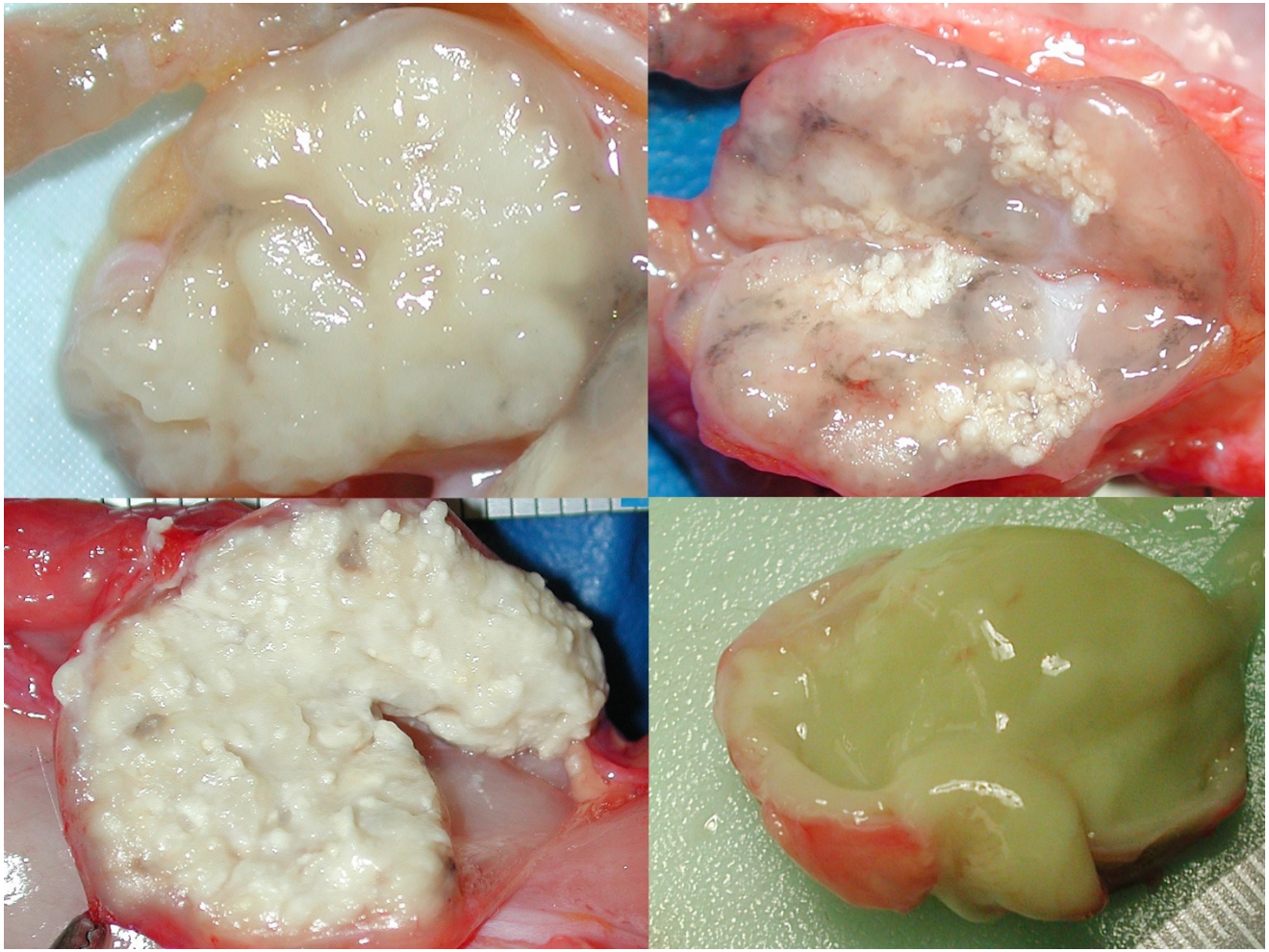
The remainder of the document primarily provides general illustrations of the lesion types listed. There is some minor additional descriptive language for clarification and some partial delving into variations that can occur. But overall, it is simply meant to illustrate some good, general examples for each of the classes – even though it is not possible to demonstrate the extensive range of variation that can occur.

As we move forward with the nuts and bolts of doing such scoring, issues may arise that force us to rethink and/or modify some of what has been proposed. Other questions will doubtlessly arise, both general and specific (for example, how do we score cluster lesions with a range of morphology, what is the working cutoff between a fibrotic granuloma and a granuloma scar as well as determining criteria as to how we parse other continuum categories, etc, etc). Guess we'll just work things out as the project progresses. Our journey begins with the first step – Here we go!

Type 1 – Necrotizing granulomas

As per our general description provided, these lesions demonstrate central necrosis with eosinophilic, amorphous acellular debris (caseum) +/- occasional stippled mineralization, concentrically surrounded by a mantle of epithelioid macrophages/multinucleated giant cells, and further peripherally marginated by lymphoplasmacytic inflammatory infiltrates. These structures often demonstrating a fibrous connective tissue capsule.

To be clear, caseum/caseation is a term that was coined for use as a gross pathological descriptor. As you all know, it is an English word borrowed from Latin for cheese. But even in a gross context that's kind of crazy. I mean how many types of cheeses are there? Does swiss cheese look like cheddar or blue cheese or cottage cheese and so on? Below is a composite I but together of a variety of necrotizing tuberculous lesions. I pretty much guarantee you that most pathologists when seeing any of them in the context of TB, would use the term "caseous" – we might qualify it with additional adjectives and descriptors, but caseous would no doubt be somehow involved in the labelling.



For better or worse however; caseation has also gotten entrenched in the histopathological description of TB – so deeply in fact, that I don't really see the convention being changes. My solution to this is to use the correct designation for such material - necrotizing – then add the term (caseous) in parenthesis behind it, when appropriate. To be clear, not all tuberculous necrosis is caseous in nature – the word typically conveys the microscopic appearance of amorphous, eosinophilic material developed because of the nature of a combination of proteins and lipids in the degenerative process. Frankly, microscopic caseum is no less variable in appearance than the gross material. We deal with our share of contributing components to this variability (neutrophils, extent of composing lipid, evolving fibrous connective tissue elements, mineral, etc). Just recognizing that "caseation" is an intrinsically variable term is half the battle. Also note, that we don't actually use the word caseous in our classification of Necrotizing granuloma types. They are Type 1a (Necrotizing active) and Type 1b (Necrotizing contained). So, let's proceed from there.

Type 1A - Necrotizing active – This designation suggests that the host has not necessarily achieved complete control or containment of the focus of infection in question. This is unquestionably a "judgment" or best guess on our part based on anecdotal knowledge of clinical disease progressions in infected animals with similar lesions of such histomorphological appearances. Active lesions generally have some or all of the following histological criteria: 1) amorphous, eosinophilic, necrotizing central debris (caseum), 2) an abundant neutrophilic component (degenerative or non-degenerative), 3) a prominent inflammatory mantle of surrounding epithelioid histiocytes (although cell-sparse mantles (generally referred to as paucicellular) are not necessarily contained lesions), 4) a tendency to extend or infiltrate beyond the defined, circumscribed outer margins and into adjacent lung parenchyma – what I generally term local alveolar extension/infiltration and 5) invasion/infiltration into the walls of adjacent vessels or bronchi, including transmural extension, as well as the presence of granulomatous inflammation within lymphatic channels. Active necrotizing lesions can also often be defined (at least in part), by what they lack. In general, they do not have an extensively dense surrounding cuff of lymphoplasmacytic cells or thick, maturing bands of peripheral connective tissue margination or extensive central fibroplasia and collagen deposition or abundant mineralization of their necrotic matrix.

Possibly one of the biggest variables in lesions considered Necrotizing active, is the extent of their neutrophilic component. Although not in use for this classification system, I often employ the descriptor degenerative neutrophil-rich for granulomas with a high number of degrading neutrophils within. Keep in mind also that when neutrophils die, they undergo pyknosis, karyorrhexis and karyolysis – fading into the eosinophilic amalgam that makes up the caseous core. So absence of visible neutrophils may only represent the lesion stage and does not necessarily mean they were not once there.

Figures 1 & 2 – Necrotizing active lesions with an abundant central degenerative neutrophilic component

Figure 3 - Necrotizing active lesion with more homogeneous, eosinophilic, neutrophil-poor central matrix. Note also the prominent thickness of and epithelioid cellularity of the inflammatory mantle (another characteristic of active lesions)

Figures 3A, 3B & 3C all illustrate a characteristic referred to as local alveolar extension, where central granuloma elements of one sort or another extend beyond the marginating boundaries of the structure, infiltrating into adjacent (alveolar) airspace. This is another common manifestation of Necrotizing active (1A) lesions

Figures 3D & 3E demonstrate infiltration into the walls of adjacent vessels or bronchi – a microscopic feature of Necrotizing active lesions

Figure 3F – Granulomatous inflammation within (and disseminating via) a lymphatic channel

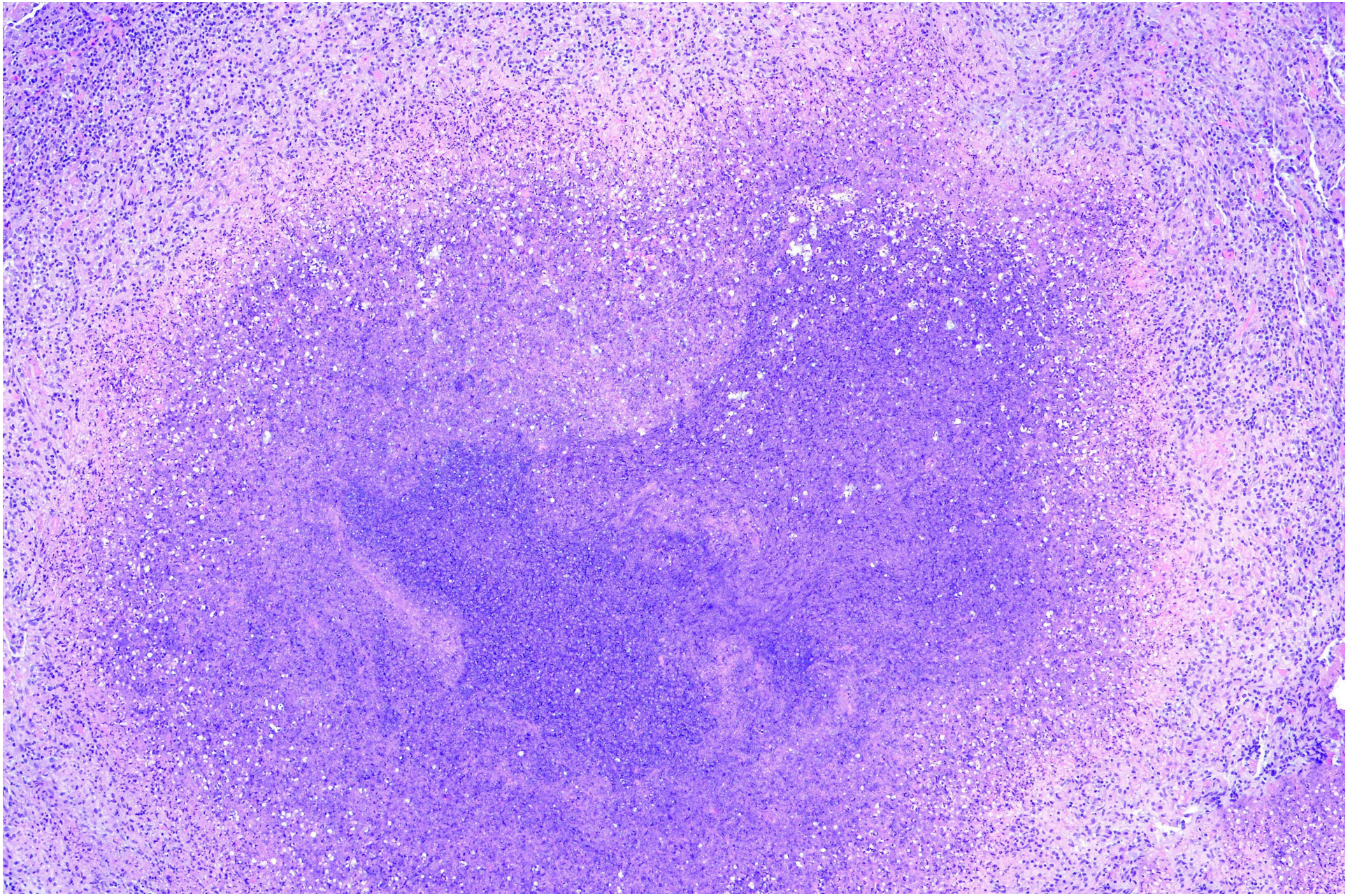


Figure 1

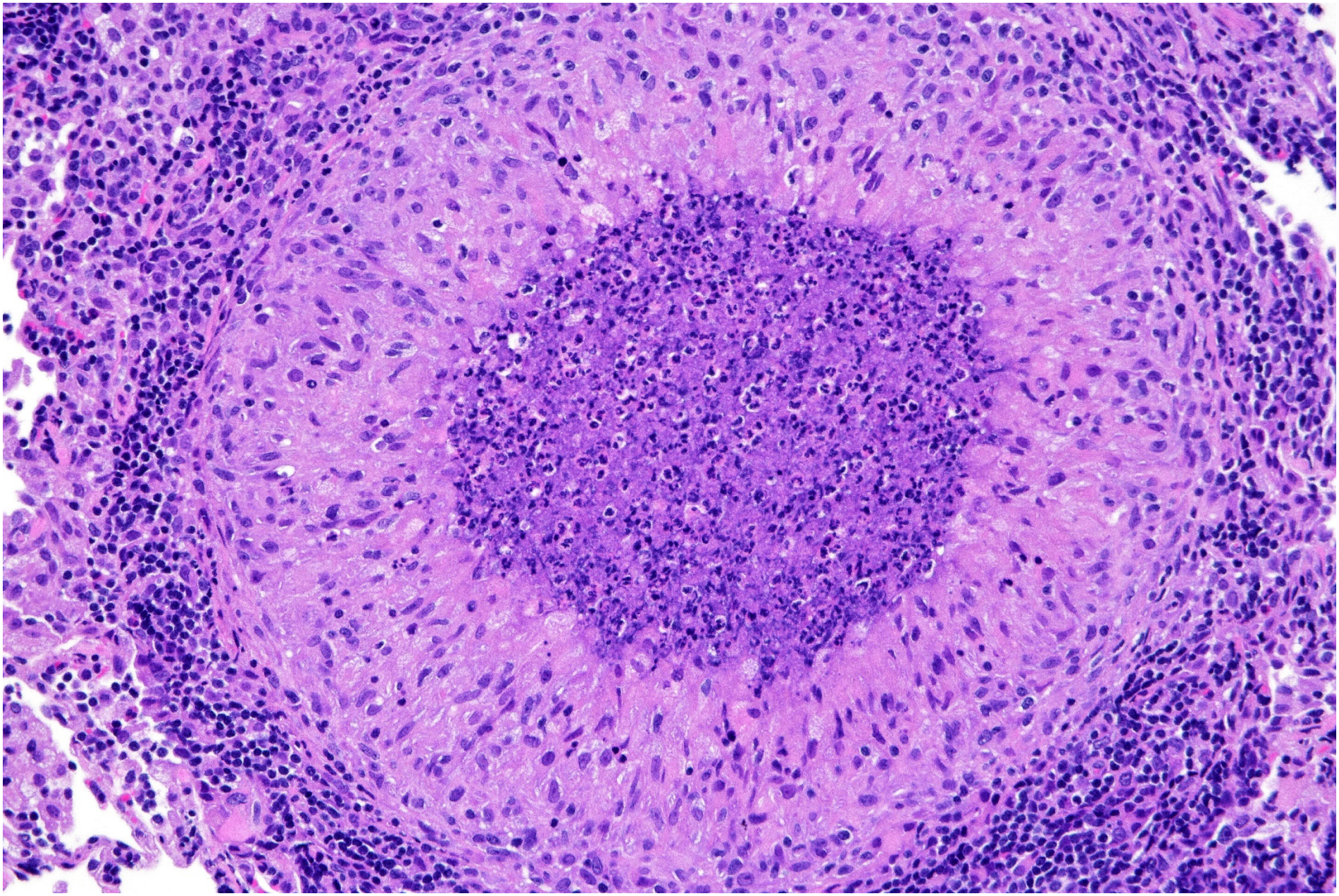


Figure 2

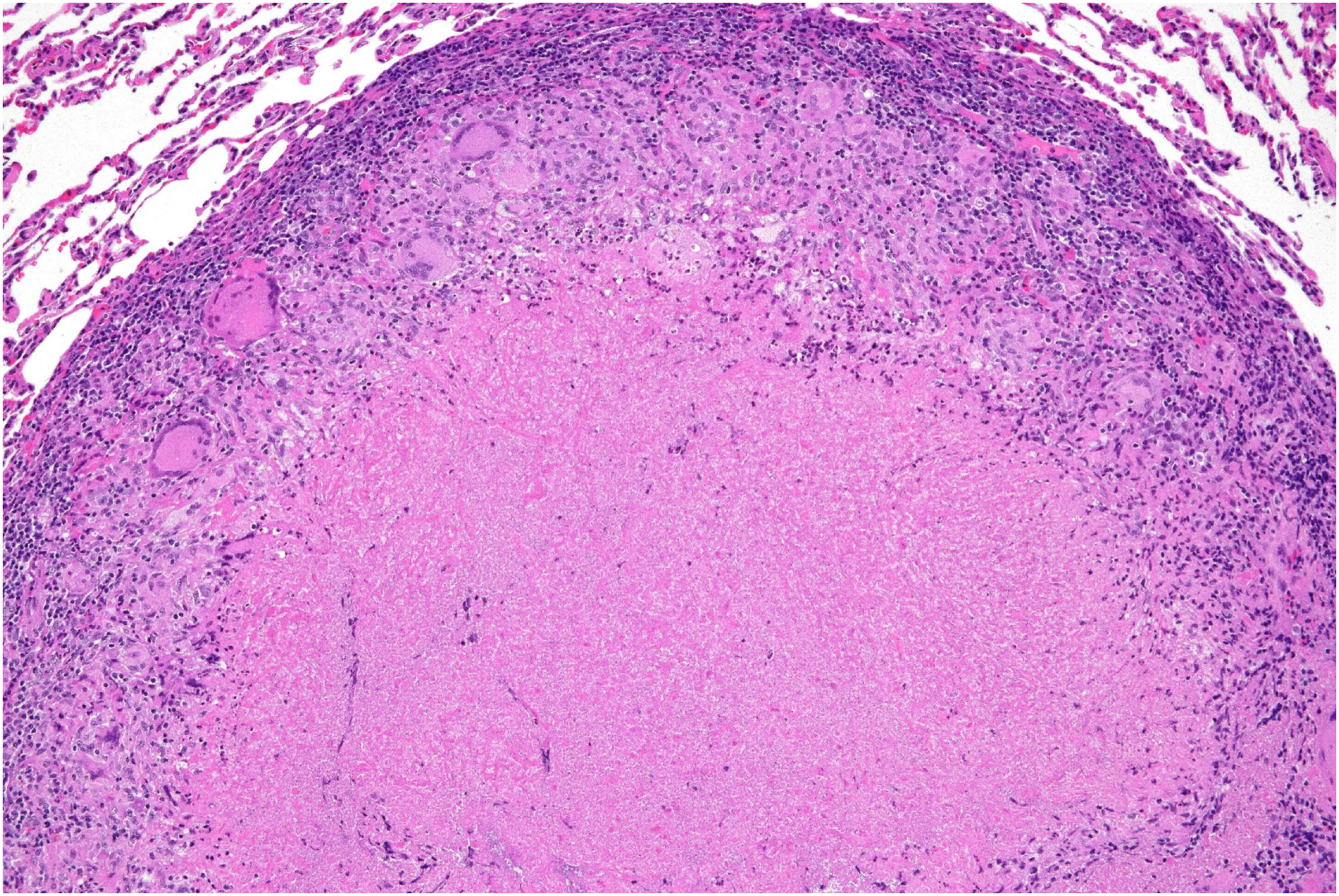


Figure 3

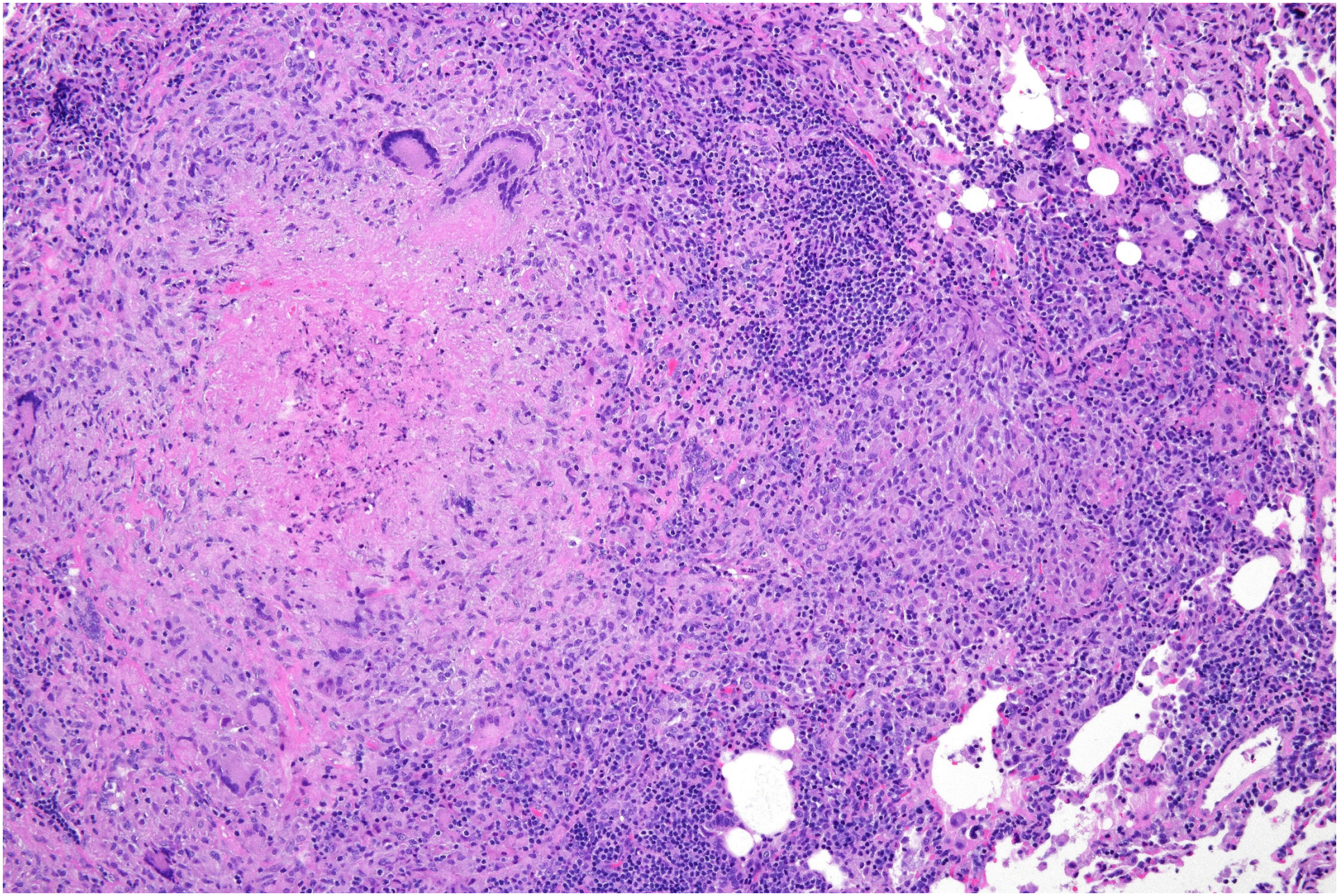


Figure 3A

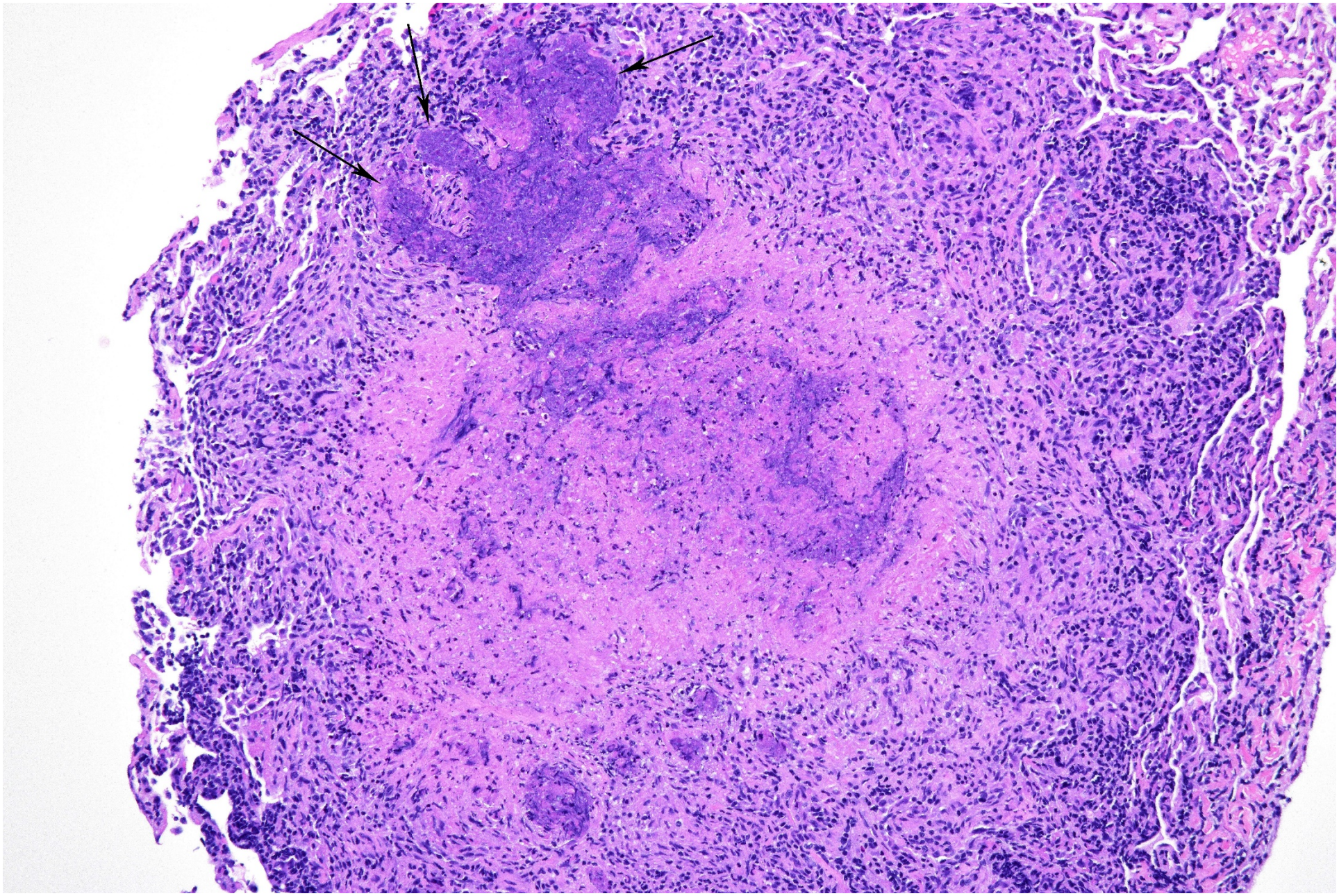


Figure 3B

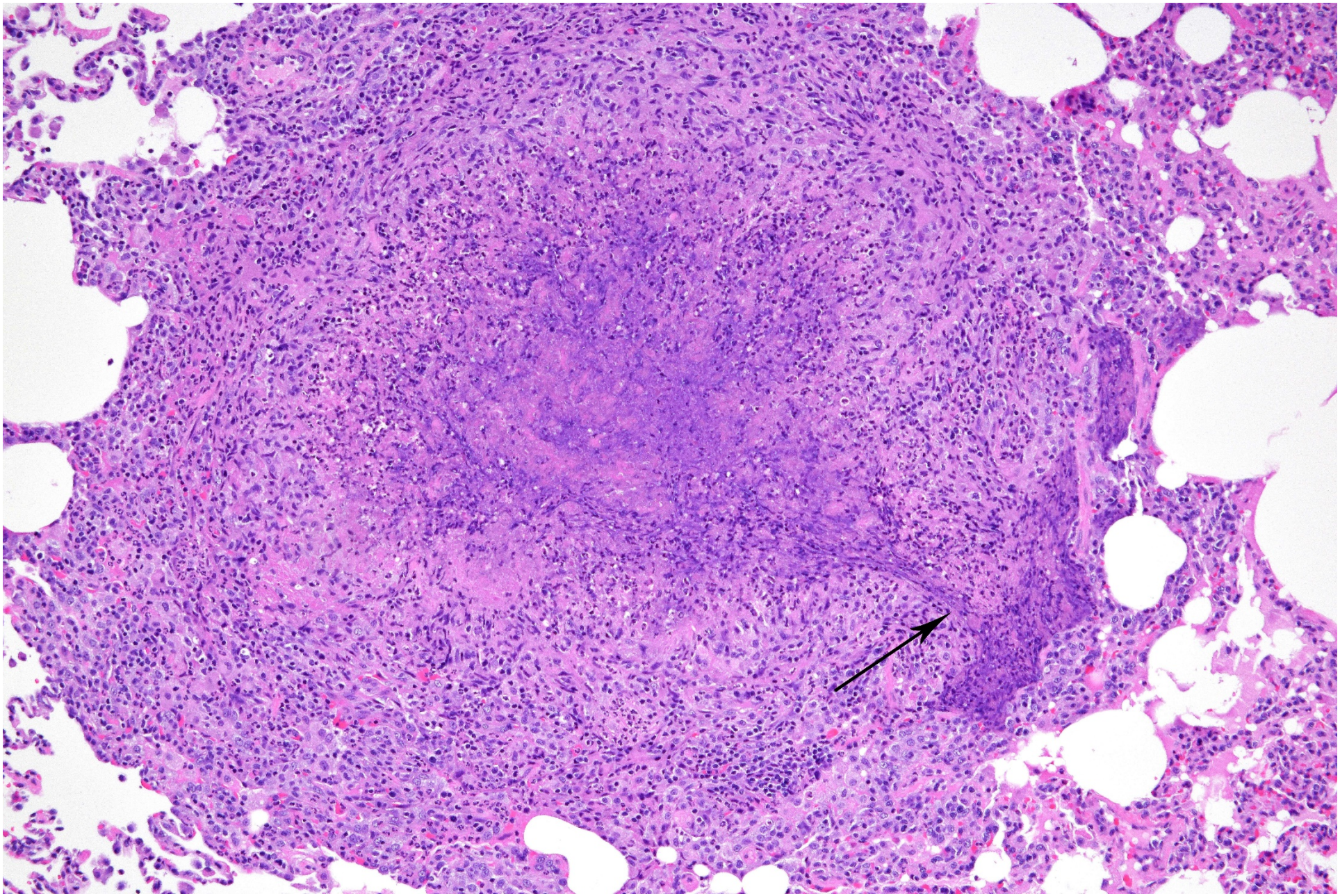


Figure 3C

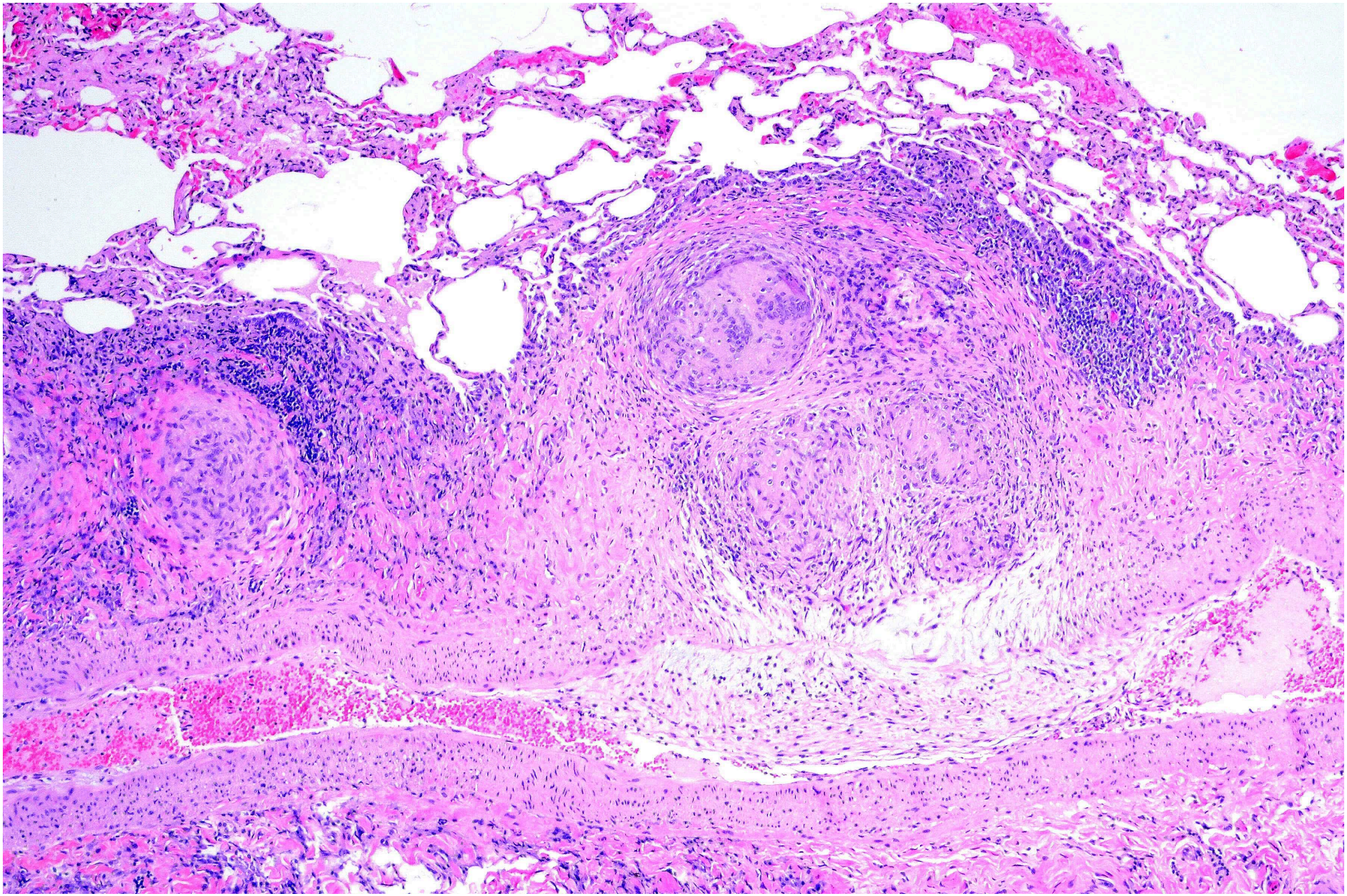


Figure 3D

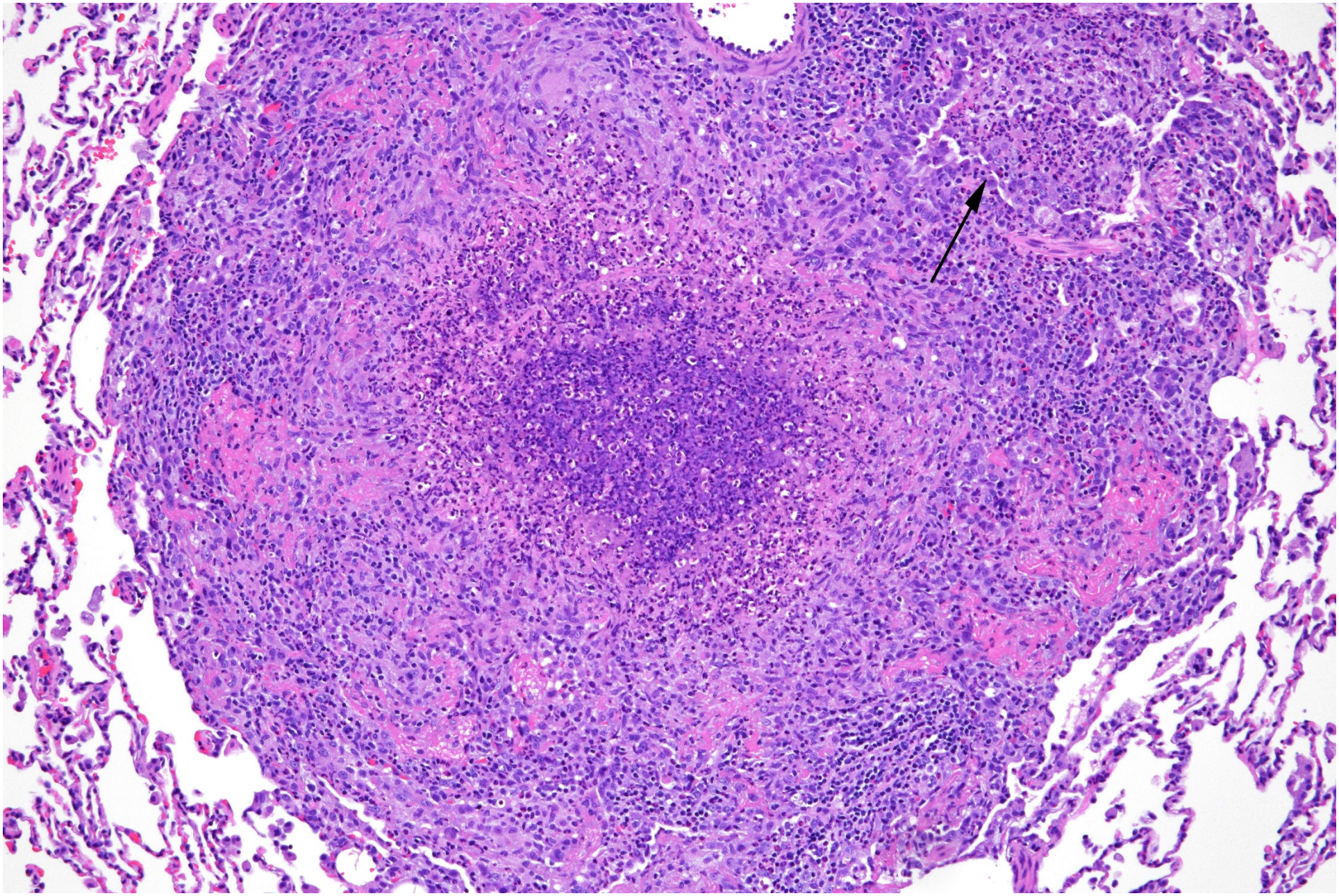


Figure 3E

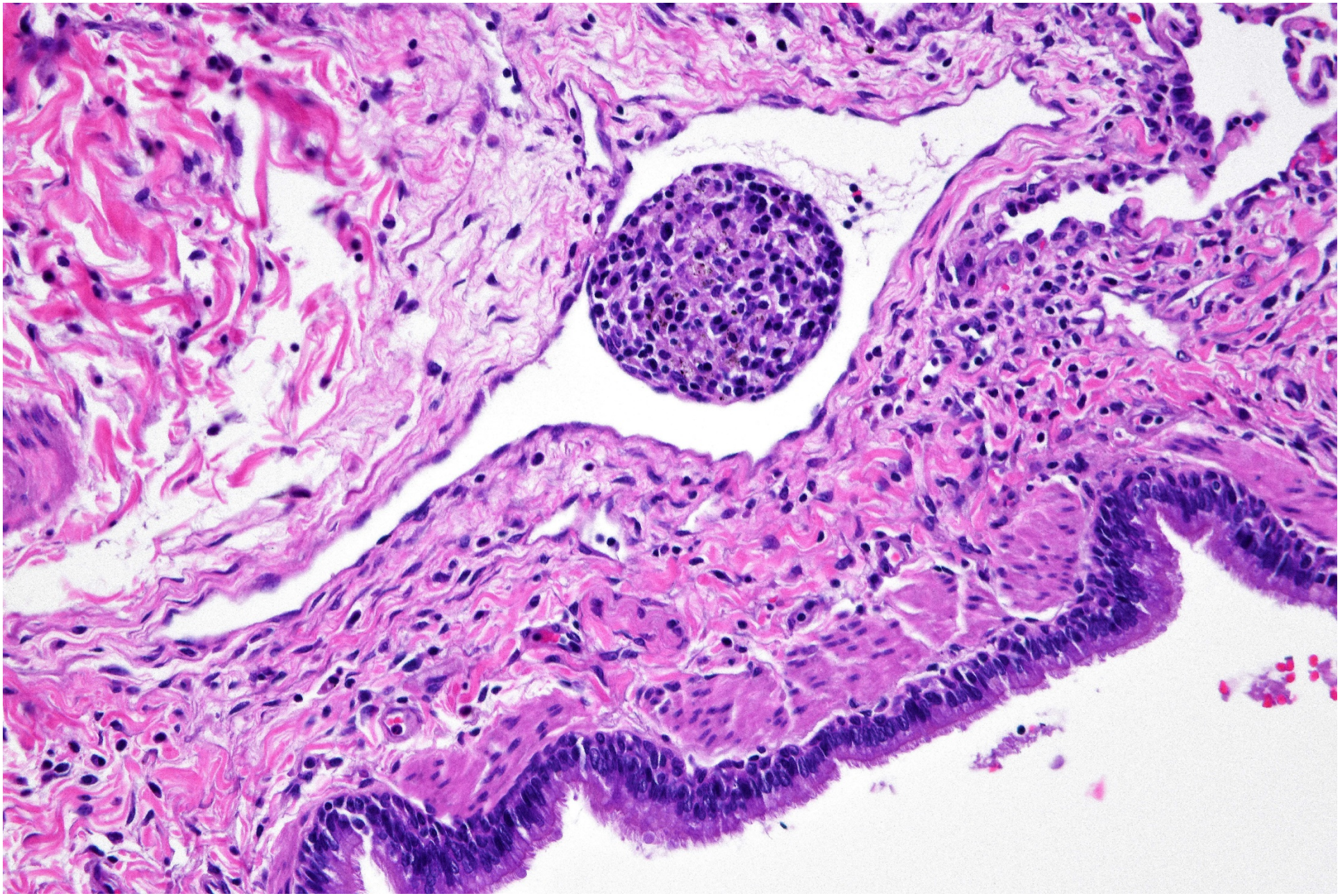


Figure 3F

Type 1B - Necrotizing contained – As opposed to the necrotizing active classification, this designation suggests that the host has achieved somewhat better control/containment of the focus of infection in question. Again, this also is a subjective “judgment” or best guess on our part based on an anecdotal perspective. Necrotizing contained lesions generally have dissimilar or opposite features of their “active” counterparts: 1) While retaining amorphous, eosinophilic, necrotizing central debris (caseum), it usually has a more “organized” appearance histologically 2) Neutrophilic components (degenerative or non-degenerative) are often less prominent (although commonly still present), 3) The inflammatory mantle often contains less epithelioid histiocytes and more cells with evidence of spindloid transformation, 4) Contained lesions in general (necrotizing or non) are typically well marginated and without disease extension into adjacent lung parenchyma, bronchial or vascular structures and 5) Most importantly, they tend to contain a prominent amount of central and/or peripheral collagen deposition/fibrosis. Mineralization of the central necrotic matrix when present is also often more extensive. Depending on stage, the surrounding lymphoplasmacytic cuff may be more prominent.

To re-emphasize, the most important histological feature in necrotizing contained lesions is the presence of abundant fibroplasia within a residual necrotic (caseous) core – or conversely, extensive mineralization of this matrix. As discussed in the foreword, containment can be part of the continuum to healing and as such, these may require differentiation from Fibrotic granulomas (Type 4 lesions). There is no hard and fast rule for making such distinction, but consider that when there remains a predominance of necrotic debris comprising the granuloma (in conjunction with the features mentioned above), it will likely warrant a contained necrotic designation, whereas if the scarring/fibrosis is the predominant histological feature (while yet retaining some evidence of granuloma substructure), a fibrotic granuloma is more appropriate. Finally, if granuloma substructure is completely replaced by dense, mature connective tissue, especially when present in an irregular, non-circumscribed shape, this defines a (resolved) granuloma scar

Based on this continuity and the discussion above, it makes sense to illustrate Necrotizing contained (Type 1B) lesions and Type 4 (Fibrotic/Healing) lesions together.

Figure 4 would be considered a Necrotizing contained granuloma in our system. Note the abundant residual necrotic matrix (caseum), yet the presence of large amounts of dense peripheral fibrosis as well as evolving central fibroplasia (arrow).

Figure 5 likewise would be classified as Necrotizing contained. Despite the prominent residual central necrotic core present, there is very heavy, dense peripheral fibrosis circumscribing the lesion.

Figure 6 is also another Necrotizing contained lesion. Even with the prominent residual neutrophil-rich necrotic core, there is abundant evolving and maturing fibroplasia present centrally. Note also the very prominent, dense surrounding lymphoid cuff.

Figure 7 is a somewhat more progressed example of a healing lesion and a good case in point of a lesion transitioning from Type 1B to Type 4A. There is definitely abundant fibrous transformation/scarring here, but because there is still ample recognizable necrotic debris and a preponderance of granuloma architecture/elements (albeit distorted) versus the scarring component, this would I believe still warrant classification as a Necrotizing contained (Type 1B) lesion.

Figures 8 & 9 demonstrate what are considered Fibrotic granulomas (4A) – these structures while retaining some very small amount of granuloma morphology & cellularity as well as their original circumscribed round/ovoid shape, are predominantly comprised of evolving dense fibrous connective tissue. As such they clearly fall within the Fibrotic granuloma category.

Figure 10 is an example of a Granuloma scar. There is no remaining recognizable granuloma structural organization, just a mature collagenous scar with abundant scattered and nodular residual lymphoplasmacytic cells (due to persistent antigenic drive). Additionally, the lesion has a distinctly irregular shape/contour.

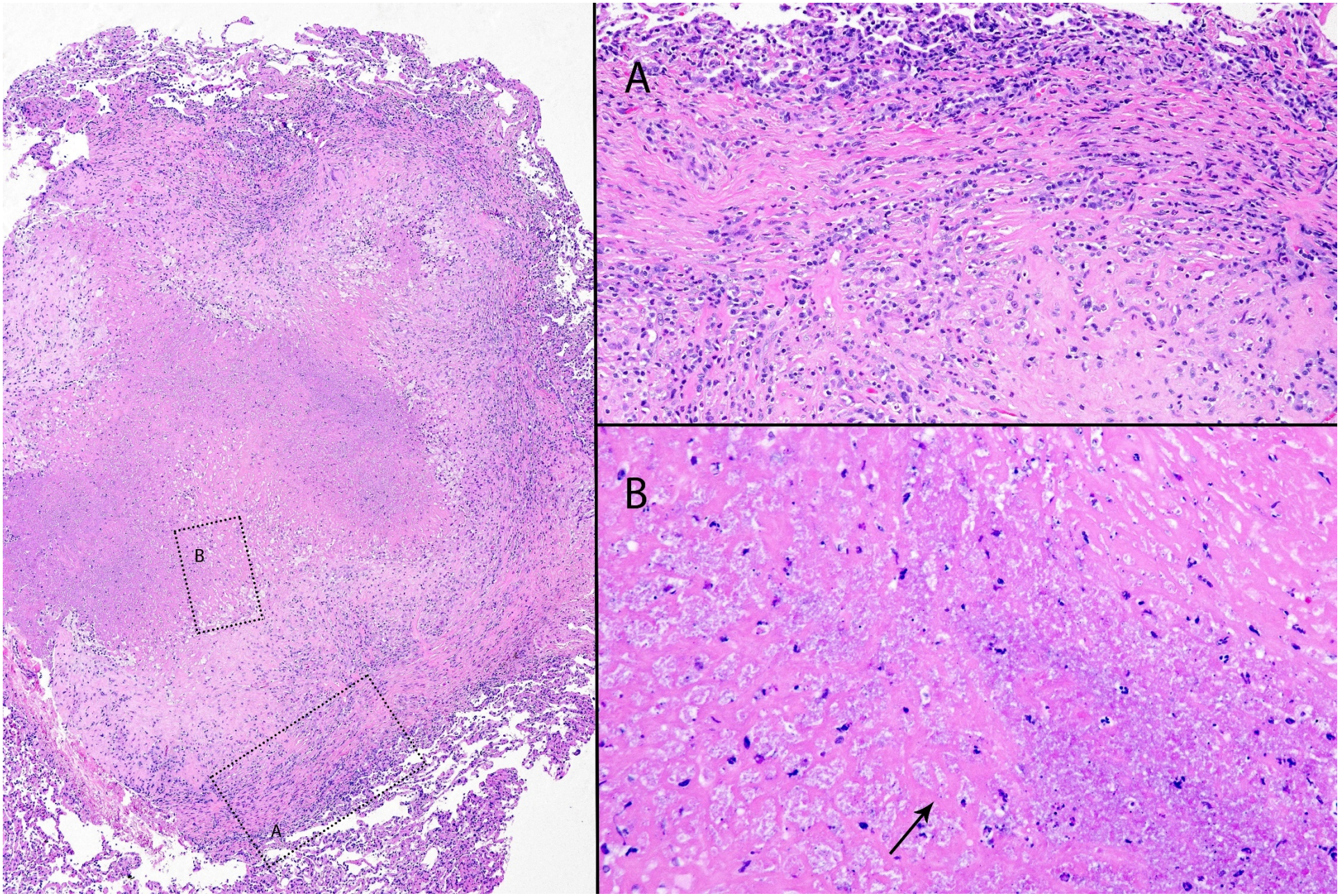


Figure 4

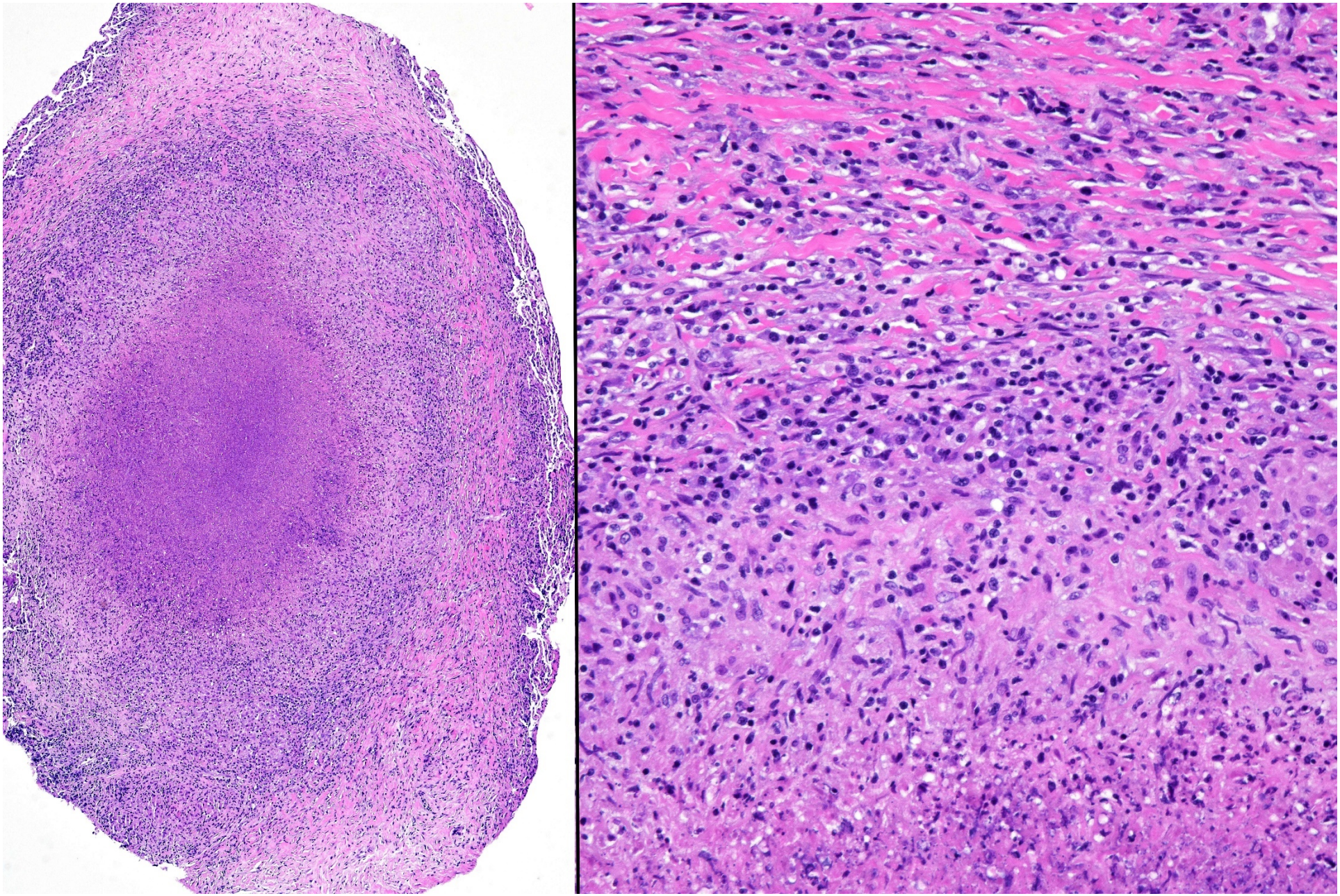


Figure 5

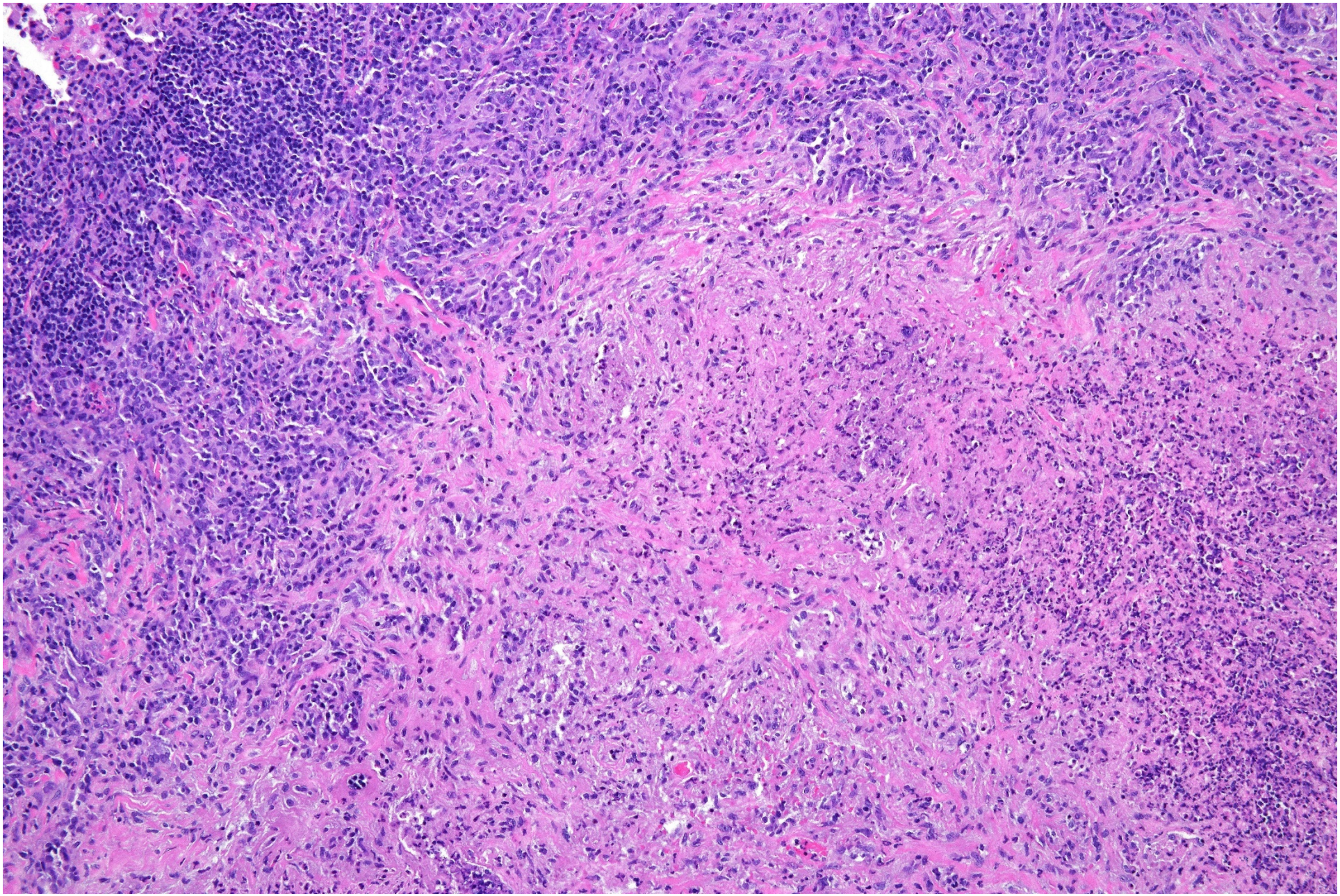


Figure 6

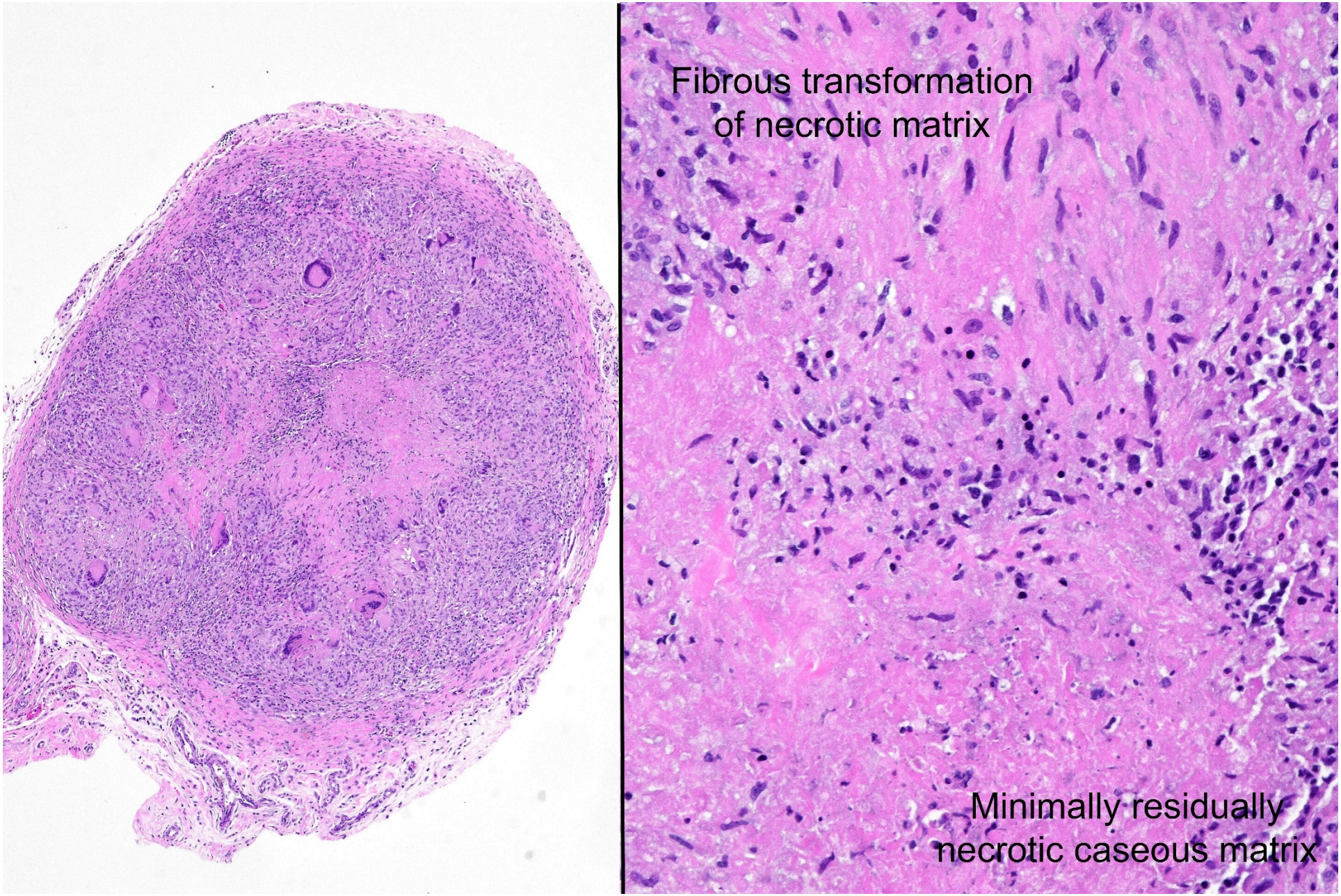

Fibrous transformation
of necrotic matrix
Minimally residually
necrotic caseous matrix

Figure 7

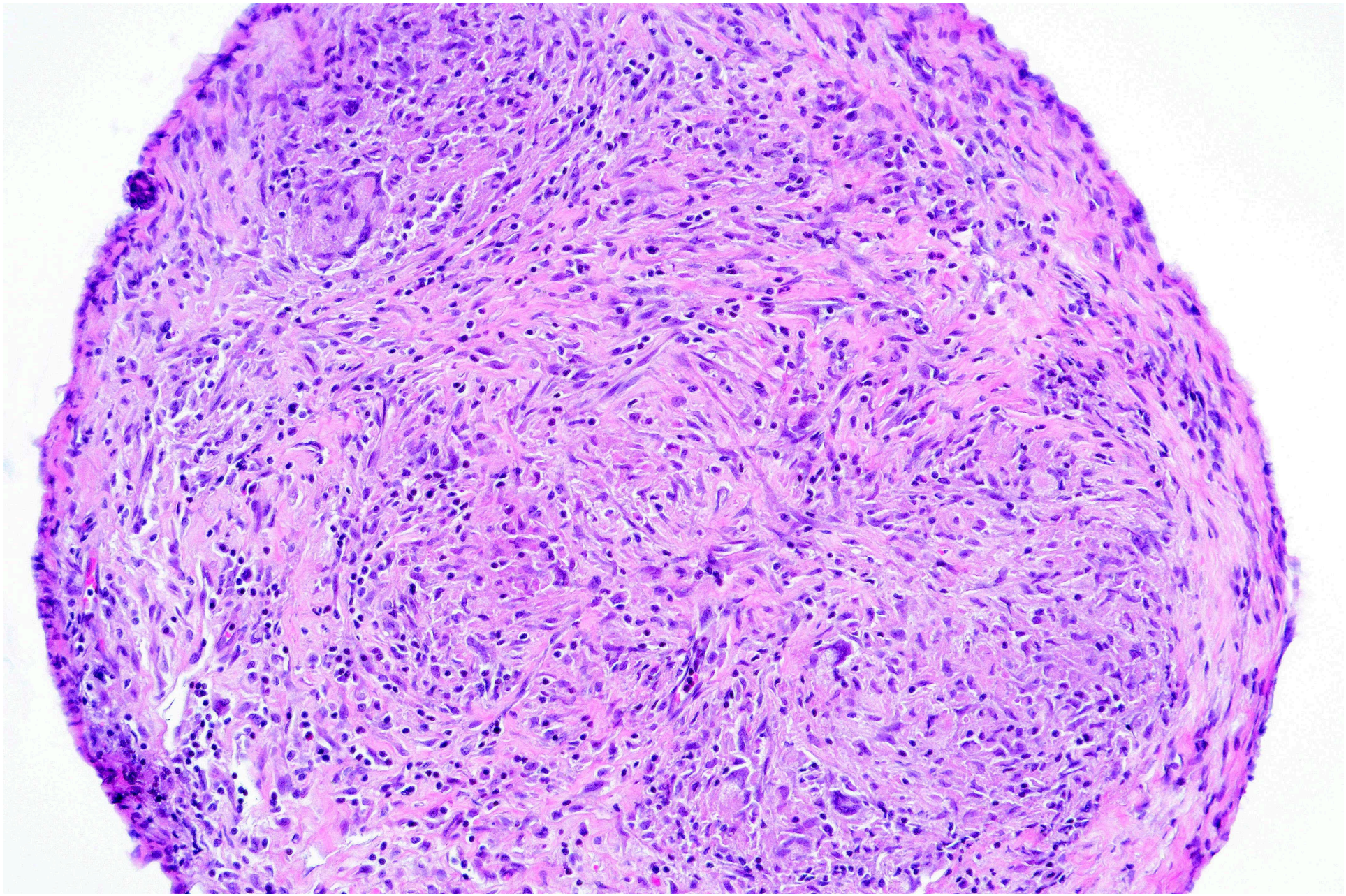


Figure 8

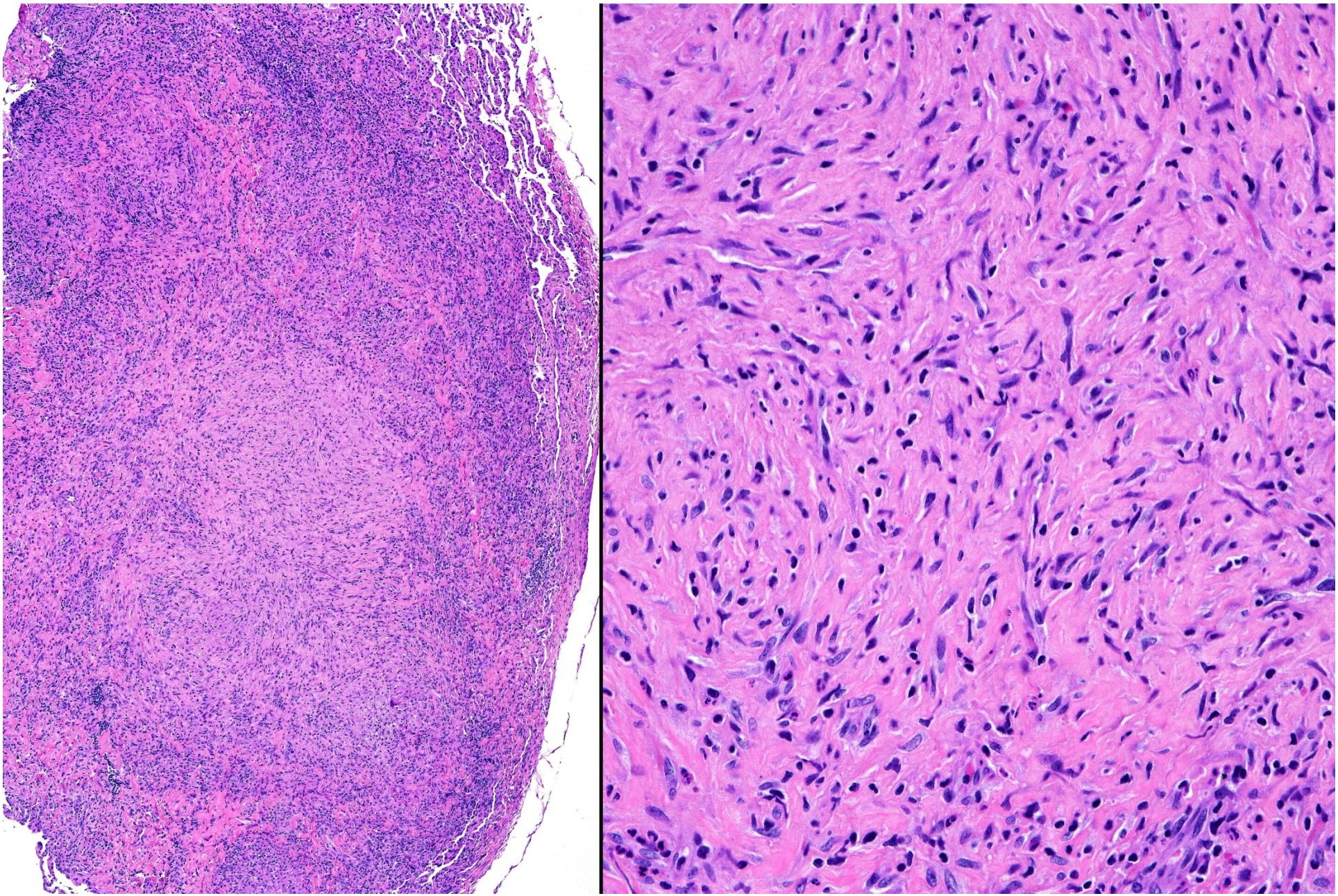


Figure 9

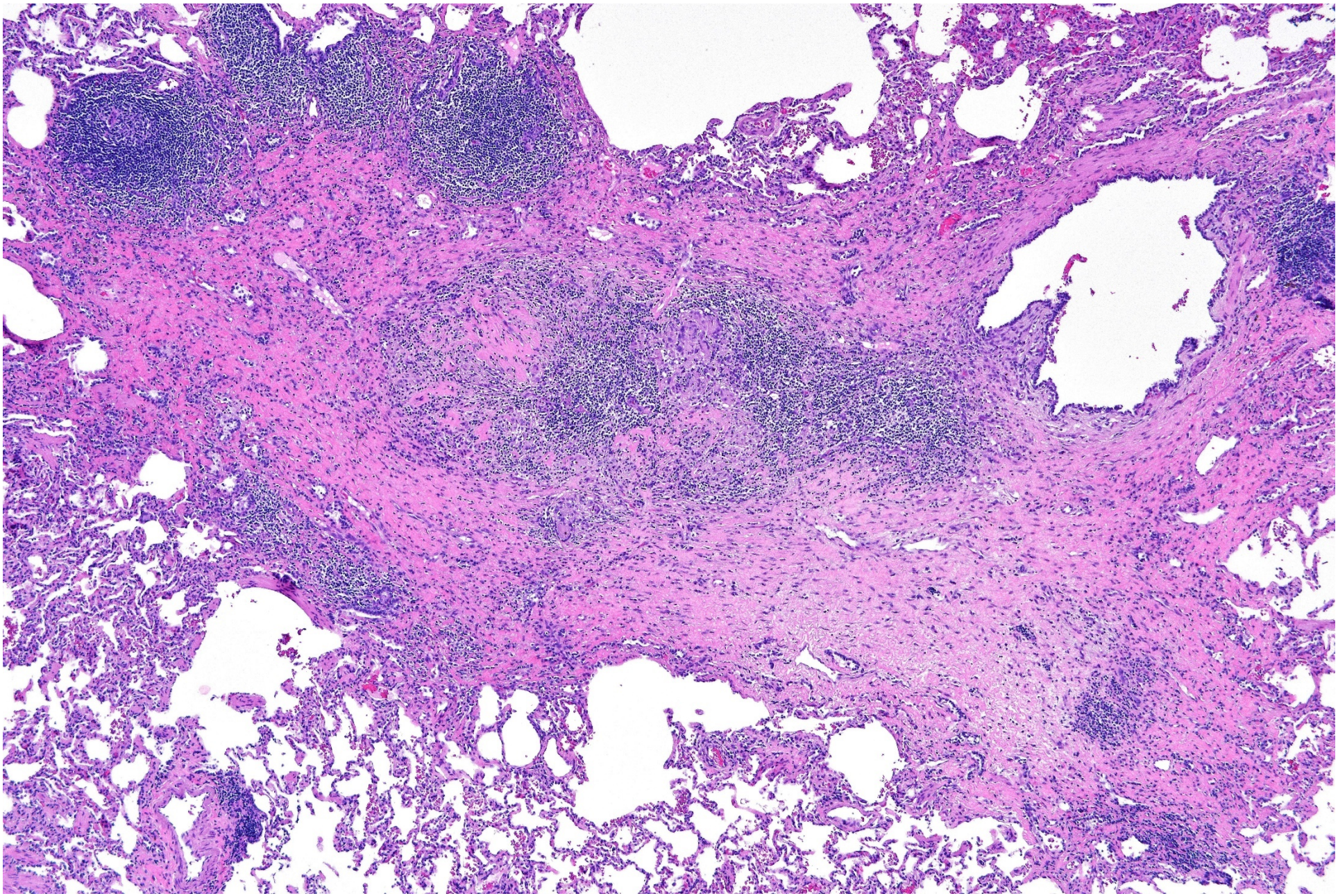


Figure 10

Moving on to Type 2 Lesions – Non-necrotizing granulomas. The obvious difference between these structures and necrotizing granulomas, is (duh) the lack of necrosis. They are still circumscribed aggregates of inflammatory cells (generally histiocytic). And they still demonstrate an architectural organization, although without a necrotic component and the various zones and cellular types associated therein, their architecture is generally less complex. Much of what applies to distinguishing active vs contained necrotizing granulomas can be used to discern these two forms of non-necrotizing lesions.

Type 2A – Non-necrotizing active: Up front, be aware of the fact that these are <u>much</u> less commonly seen in the lung and much more frequently present in thoracic lymph nodes. I suspect this is because they often transition rapidly to a necrotizing state. In any event, the hallmark characteristic of non-necrotizing active foci is a composition of large, plump epithelioid histiocytic cells, often including multinucleated giant cells. They are often smaller in size than necrotizing lesions (again, I suspect that there is a critical dimension of growth at which central necrosis is an inevitable consequence). Scattered other inflammatory cells may be a component of their makeup, although generally a modest one. They may be marginated by a thin rim of fibrous connective tissue, although this is typically not substantial. And again, from an exclusionary component, they tend to have either no or modest amounts of spindloid transformation of their cellularity and/or frank collagen deposition/scarring.

Type 2B – Non-necrotizing contained: Conversely, these structures have a substantial amount of central fibrous organization or spindloid transformation of their epithelioid component. Peripheral fibrosis may be more prominent. Numerous lymphoid cells surrounding or extending into non-necrotizing granulomas is generally a good indication of evolving containment. Keep in mind that necrotizing lesions which heal, often evolve back into a non-necrotizing state. When the caseously necrotic matrix has been completely replaced by fibroblasts and connective tissue scar, the resulting lesion is by definition, non-necrotizing.

Figure 11 – This is a classic (and fairly rarely occurring) pulmonary non-necrotizing active granuloma (Type 2A). Note the abundant large epithelioid histiocytes and multinucleated cells with prominent eosinophilic cytoplasm.

Figures 12 - This is also a non-necrotizing granuloma by virtue of the lack of observable necrosis. It appears to be comprised of coalescing nodular subunit structures. Additionally, there is a somewhat more prominent infiltrate of peripheral lymphocytes. Although the majority of cells within are epithelioid in appearance, there is some spindloid transformation of epithelioid cells (with frank fibrosis being minimal). Again, this is likely a continuum lesion and although it could reasonably be placed in either the 2A or 2B group, I believe it probably warrants the former classification (2A).

Figure 13 is an example of a non-necrotizing granuloma that clearly falls in the 2B grouping. Note the abundance of evolving fibrosis, the large infiltrating lymphoplasmacytic component and the extensive spindloid transformation of the residual cells.

The same criteria that were discussed for differentiating Type 1B from Type 4 lesions apply to type 2B as well. In fact as noted above, healing lesions are often in a non-necrotizing state by definition, by the time they evolve into either fibrotic granulomas or granuloma scars.

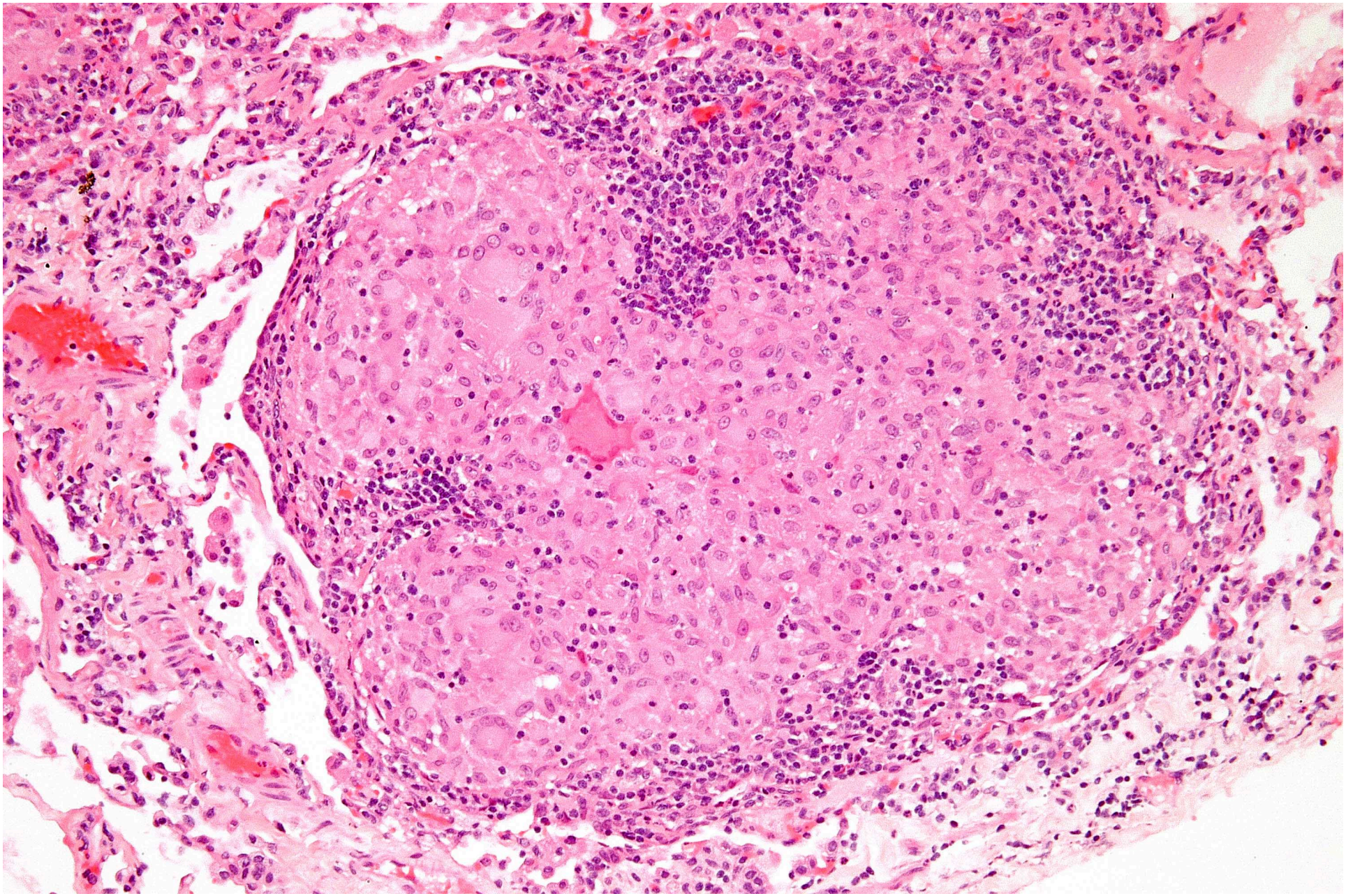


Figure 11

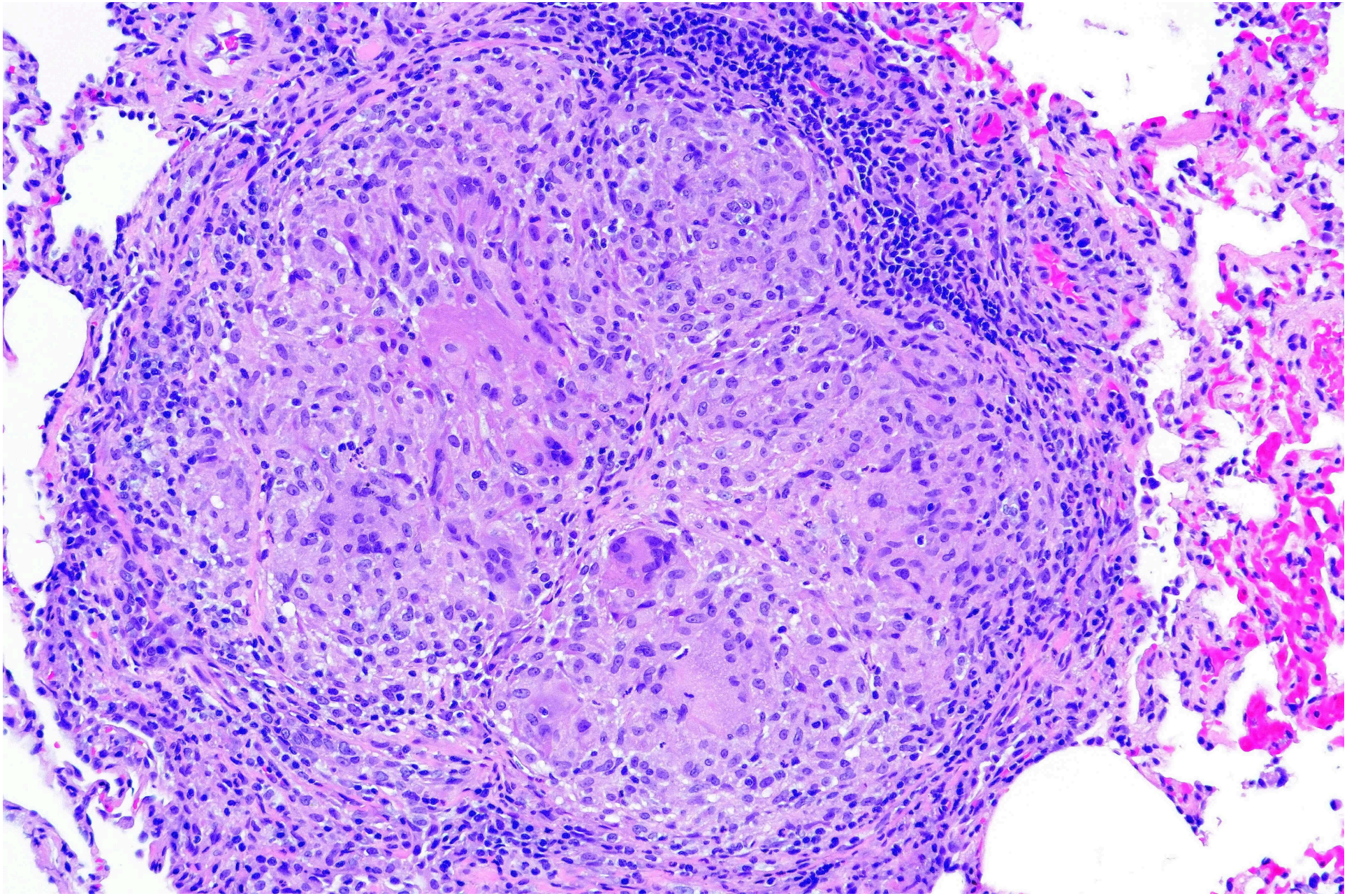


Figure 12

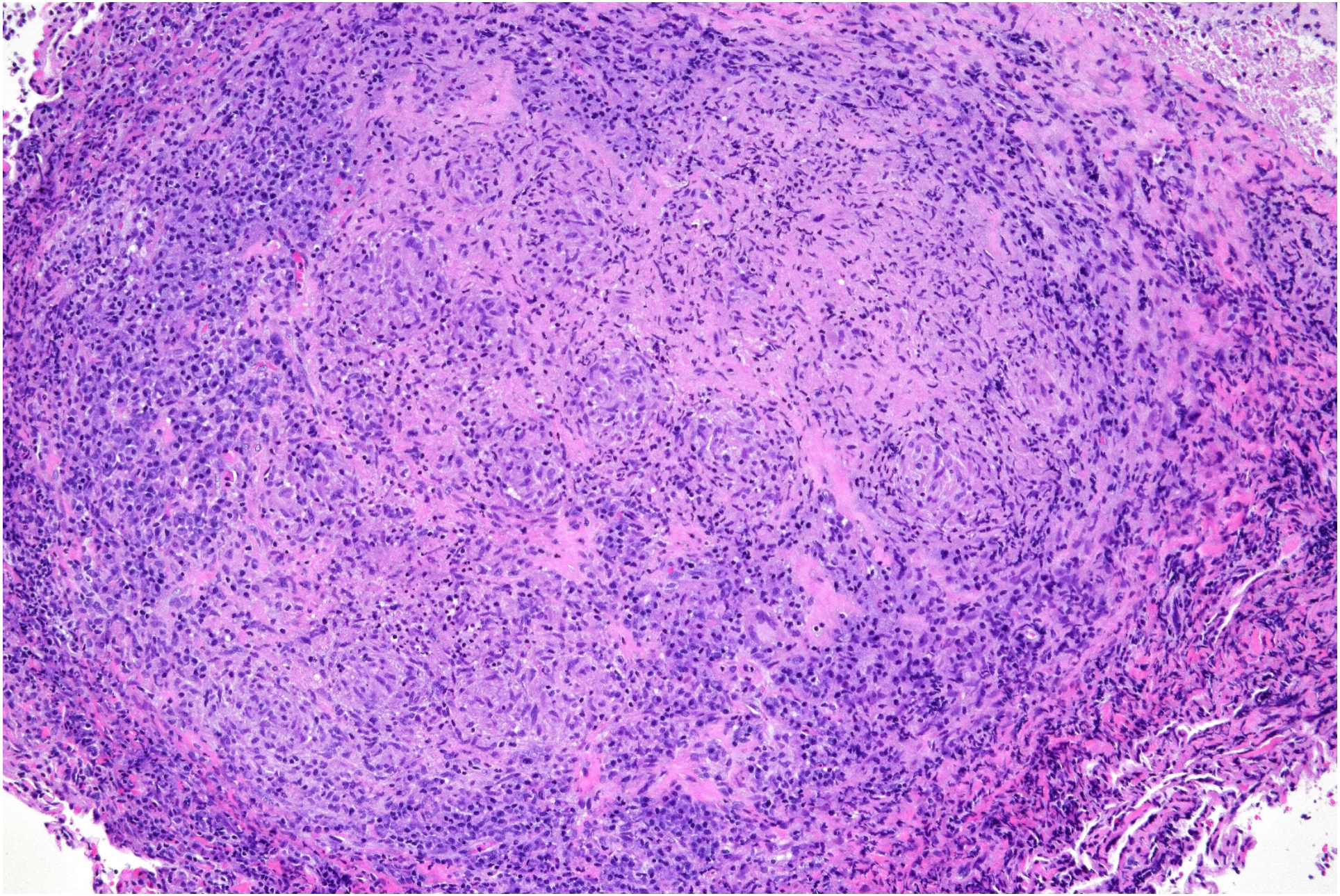


Figure 13

Type 3 Lesions – Granulomatous inflammation outside of defined granuloma structure formation

The good new with this category is that (at least for macaques), lesions within will be relatively uncommonly represented (compared to other types).

Tuberculous pneumonia (Type 3A) is likely the most common of the three lesions you might see in the context of this project and the harvested lesions we'll be reviewing. TB pneumonia can be defined either as a large area of coalescing or confluent necrotizing (caseous) granulomas or lesions in which histiocytic inflammation without frank granuloma structure extends in a direct, seemingly unimpeded fashion from alveolus-to-alveolus. Often both types of processes are present within a given area. The biggest problem with this category is deciding when a lesion qualifies as TB pneumonia vs simply a large area of coalesced granulomas. In some cases the gross appearance makes it clearly obvious that there is a focally extensive consolidating process warranting a pneumonic designation. In other lesions however, the distinction can be less clear. Once again, this demonstrates how various lesions tend to represent a continuum process. In all situations, correlation with the description of the gross lesion is definitely warranted. I have over the years adopted a very rough rule of thumb that if the dimensions of such lesions extend for >1-2 cms (in histologic section), a label of TB pneumonia is generally warranted. This is especially true if there is a large component of histiocytic alveolus-to-alveolus extension of disease.

Type 3B, Granulomatous alveolitis encompasses a range of lesions that is often seen incidentally in random lung tissue and rarely harvested as an individual scan-matched structure per se. Categorically though, it remains a form of granulomatous inflammation without distinct/definitive granuloma structure formation and as such, warrants a place in the Type 3 category. Typically, there are in a patchy fashion, alveoli containing large, activated epithelioid macrophages and/or fibrinoid necrotic debris. The size/extent of these lesions as well as their composition can be quite variable. Occasionally, one may encounter a focally extensive region of granulomatous alveolitis (i.e. confluent involvement of numerous alveolar structures) that has begun to "organize" architecturally into the transformation of a granuloma structure. I do believe that this is the pathophysiological basis for how many of the fully formed granulomas we see evolve into being. Again though, barring such an occasional transitional form, it is unlikely that scan-matched, harvested lesions will fall in this category.

Type 3C – Cavitation. Again, rare. It is defined as a large bronchus that has been transmurally invaded, effaced and expanded by tuberculous disease, leaving an ectatic space or "cavity", often with only a small residual portion of recognizable bronchial wall. These structures are generally filled with necrotic debris – either acellular amorphous caseum or degenerative neutrophil-rich suppurative material and would be referred to as "dirty" cavities. In contrast, "clean" cavities are the residual result of such lesions after treatment/healing. I have not yet to date, recognized a clean cavity in our work. The only caveat here is deciding when a transmurally-invaded bronchus has been expanded and effaced to the extent that a cavitary designation is warranted. Again, a framework of 1-2 cms may be a reasonable working definition.

TB pneumonia

Figure 14 – This is a gross:microscopic illustration of a large region of tuberculous pneumonia. Although structured, coalescing necrotizing granulomas are present, this particular lesion consists primarily of direct alveolus-to-alveolus extension of either histiocytic inflammation or acellular eosinophilic necrotic debris (caseum)

Figure 15 – This is a higher power of activated histiocytic infiltrates extending directly from alveolus-to-alveolus without forming granuloma structures (alveolar septae and terminal alveolar spaces are clearly visible in section). Such an appearance suggests limited host immune containment.

Figure 16 – Another gross:microscopic illustration of TB pneumonia – this pneumonic lesion being comprised of more coalescing to confluent necrotizing granuloma structures

Granulomatous alveolitis

Figure 17 – This is a focal, small nidus of granulomatous alveolitis in which histiocytic inflammation, including multinucleated giant cells, involve a single alveolar structure

Figure 18 – In this somewhat larger focus of granulomatous alveolitis, aggregates of histiocytic inflammatory cells are present in numerous adjacent alveoli. Note the prominent lymphocytic infiltrate present in the interstitial of the surrounding alveolar walls

Figure 19 – Not uncommonly, the material within the alveoli contains abundant fibrinoid necrotic debris with less prominent (or absent) cellularity, be it histiocytic or granulocytic populations. I generally still designate such less-cellular foci “granulomatous alveolitis”, even though one could argue that a descriptive term such as fibrinoid alveolar necrotic debris accumulation might be more accurate.

Figures 20 & 21 – Occasionally a larger confluent area of granulomatous alveolitis will begin to demonstrate circumscription and structural organization, such lesions may potentially evolve into granulomas per se

Cavitation

Figures 22 & 23 – These illustrations represent developed tuberculous cavities - large bronchial airways that have been transmurally invaded, effaced and expanded by disease, leaving an ectatic space, often with only a small residual portion of recognizable bronchial wall. Identifying cavities histologically is often dependent on correlation with the gross appearance of the harvested lesion

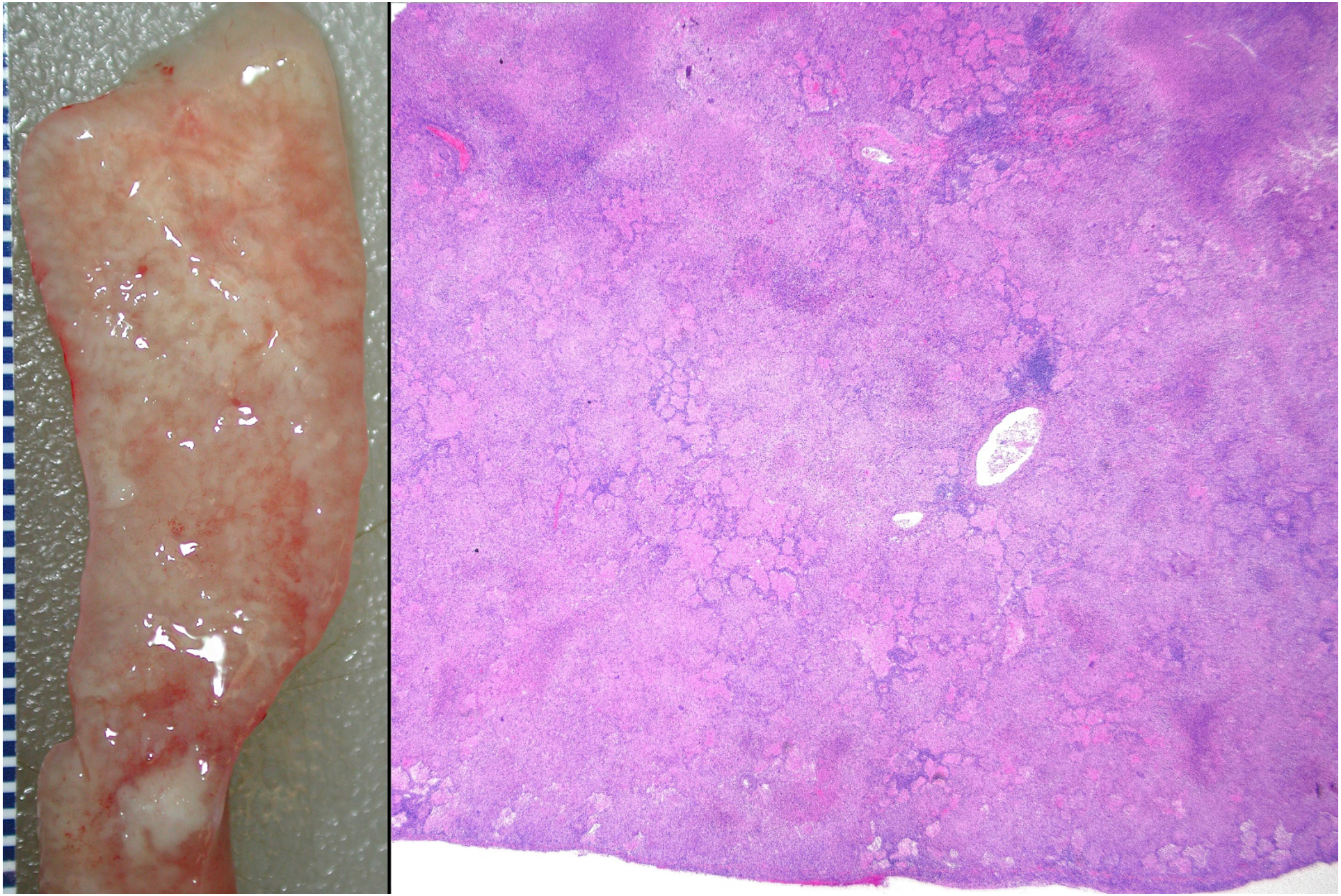


Figure 14

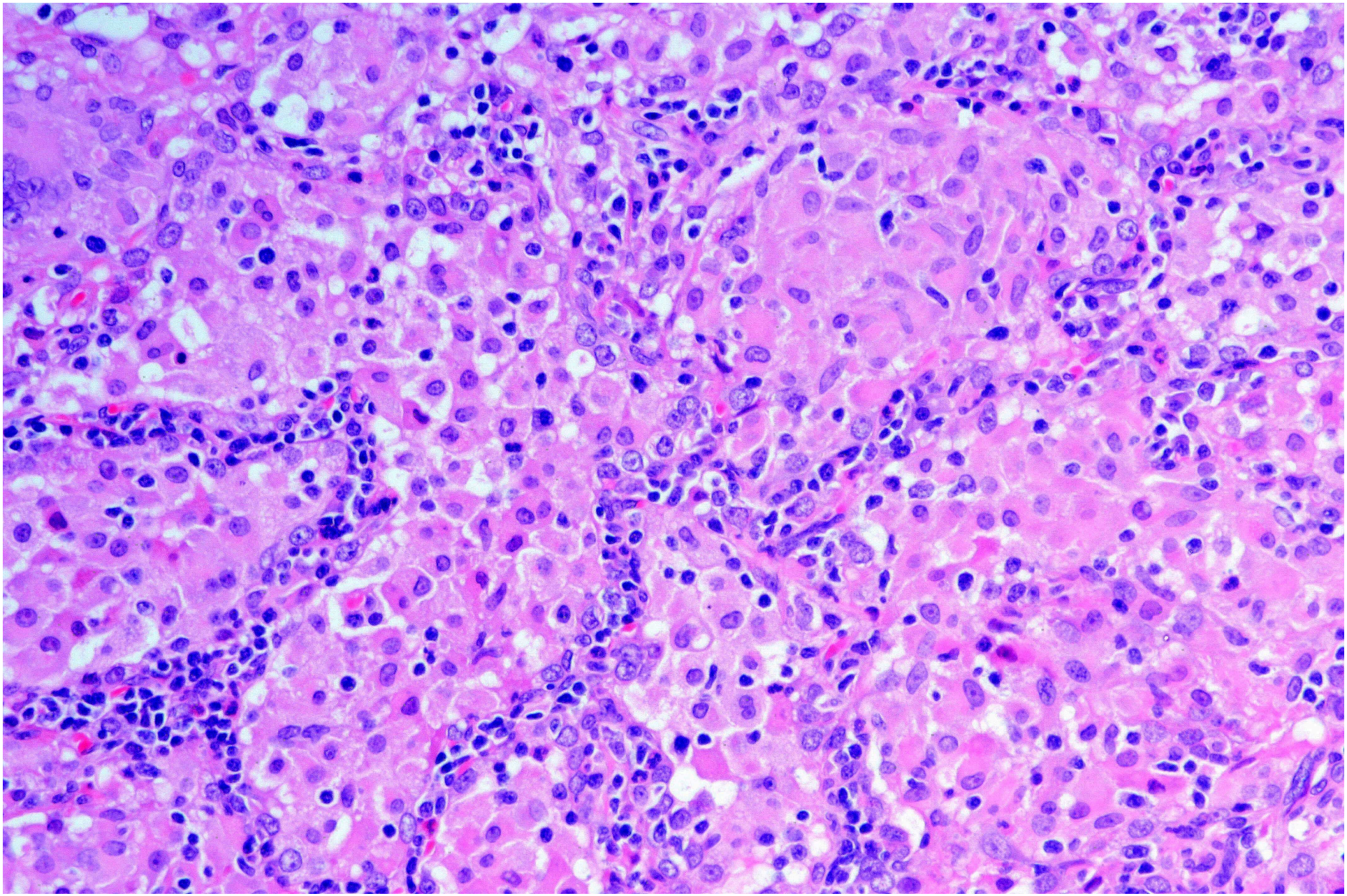


Figure 15

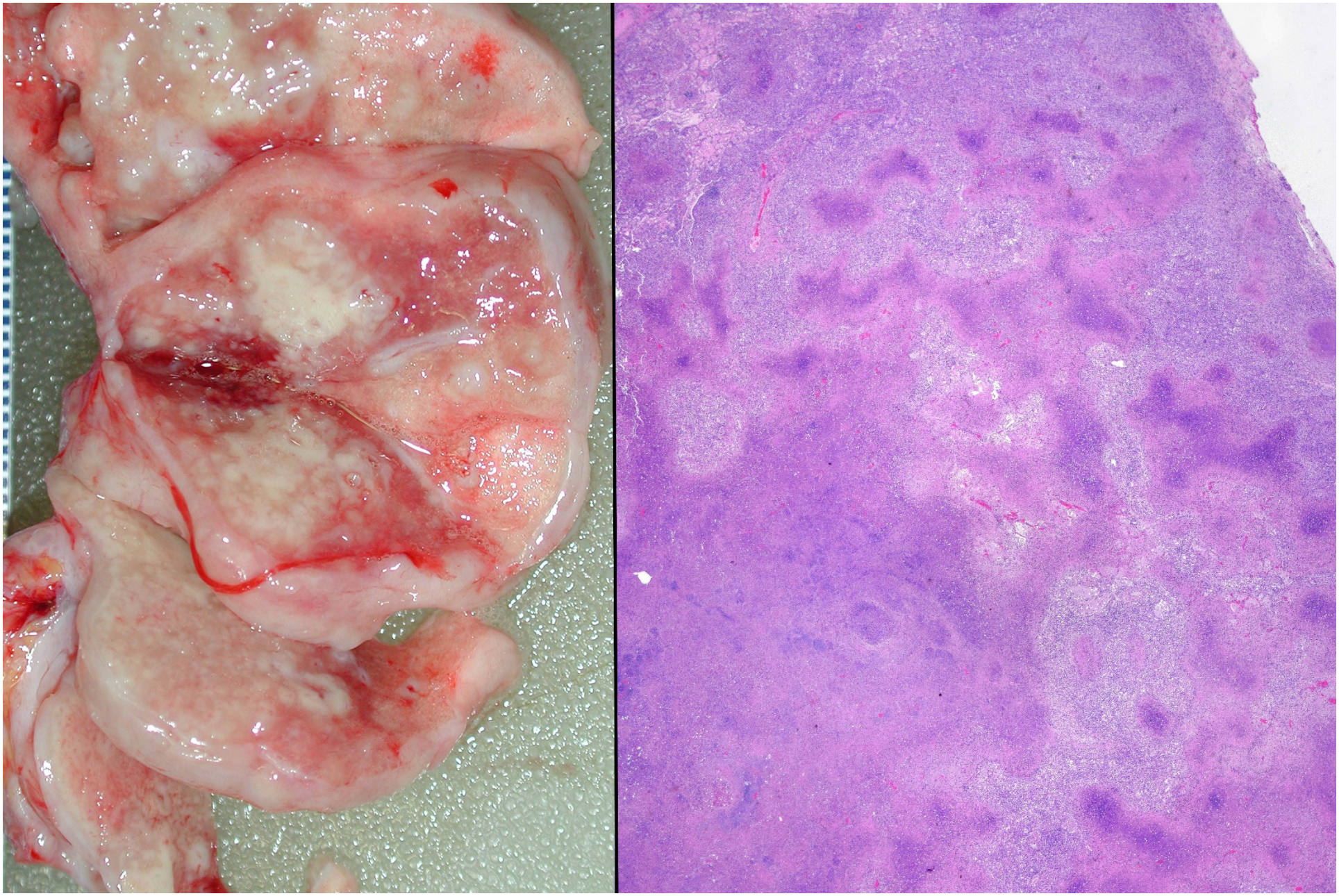


Figure 16

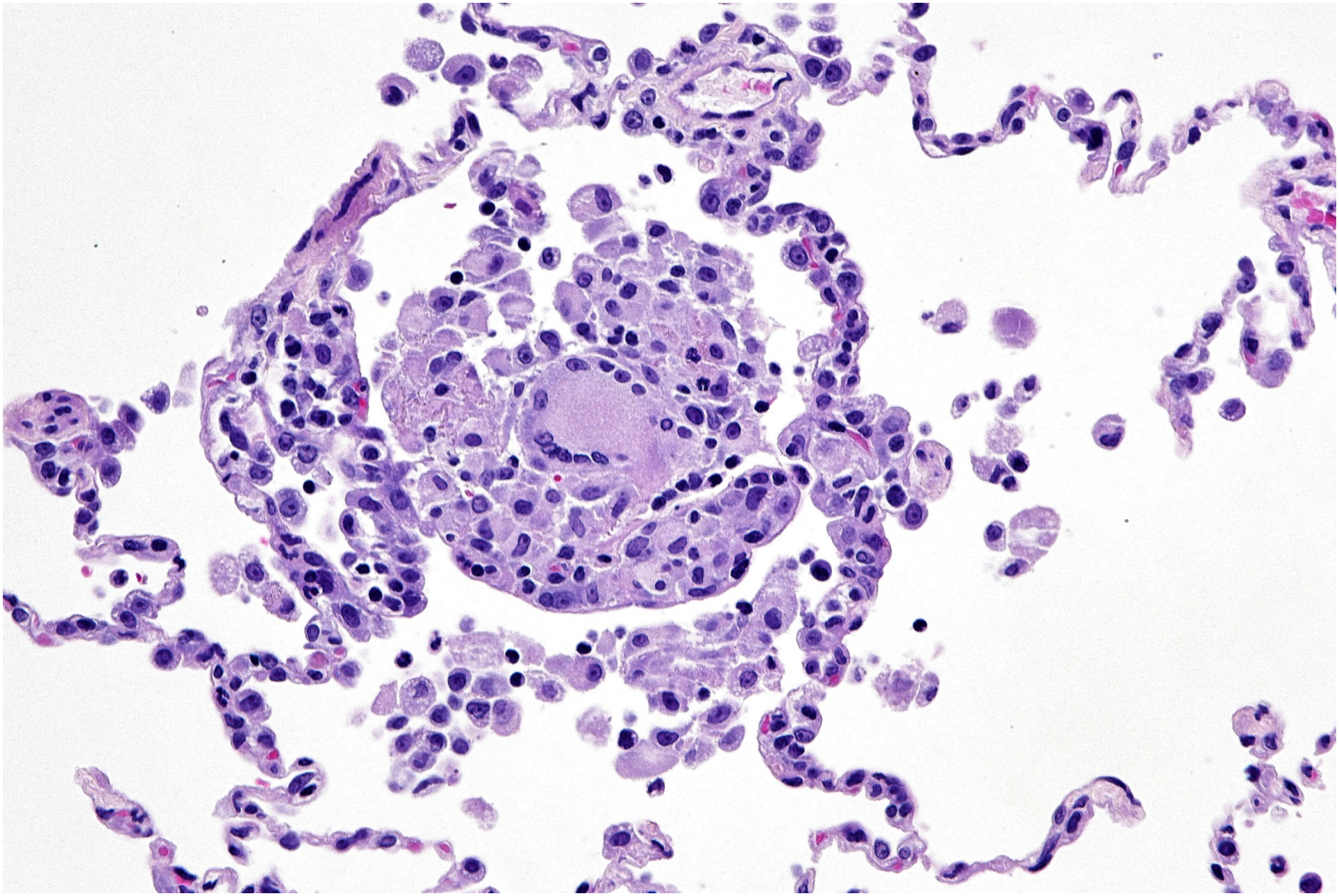


Figure 17

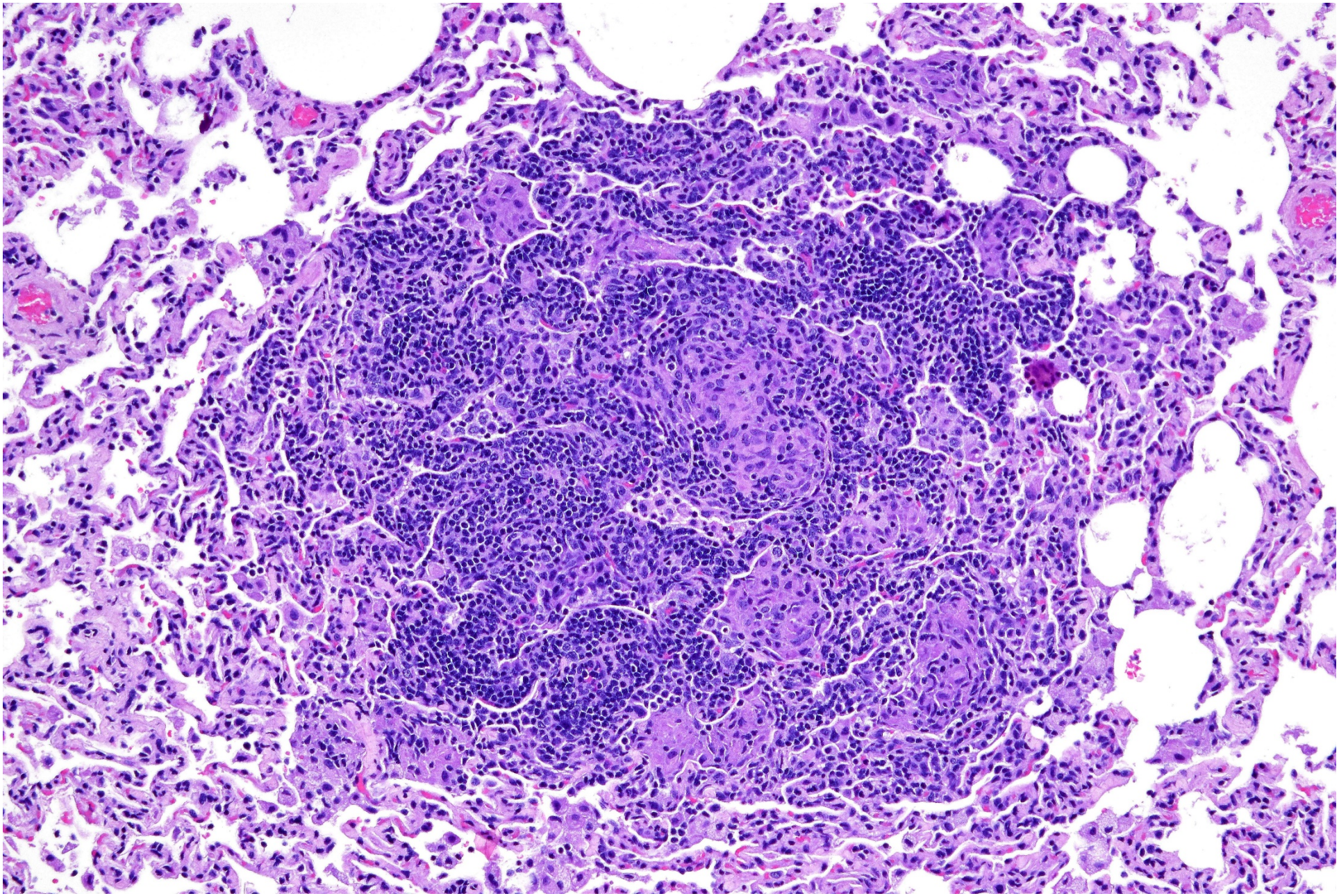


Figure 18

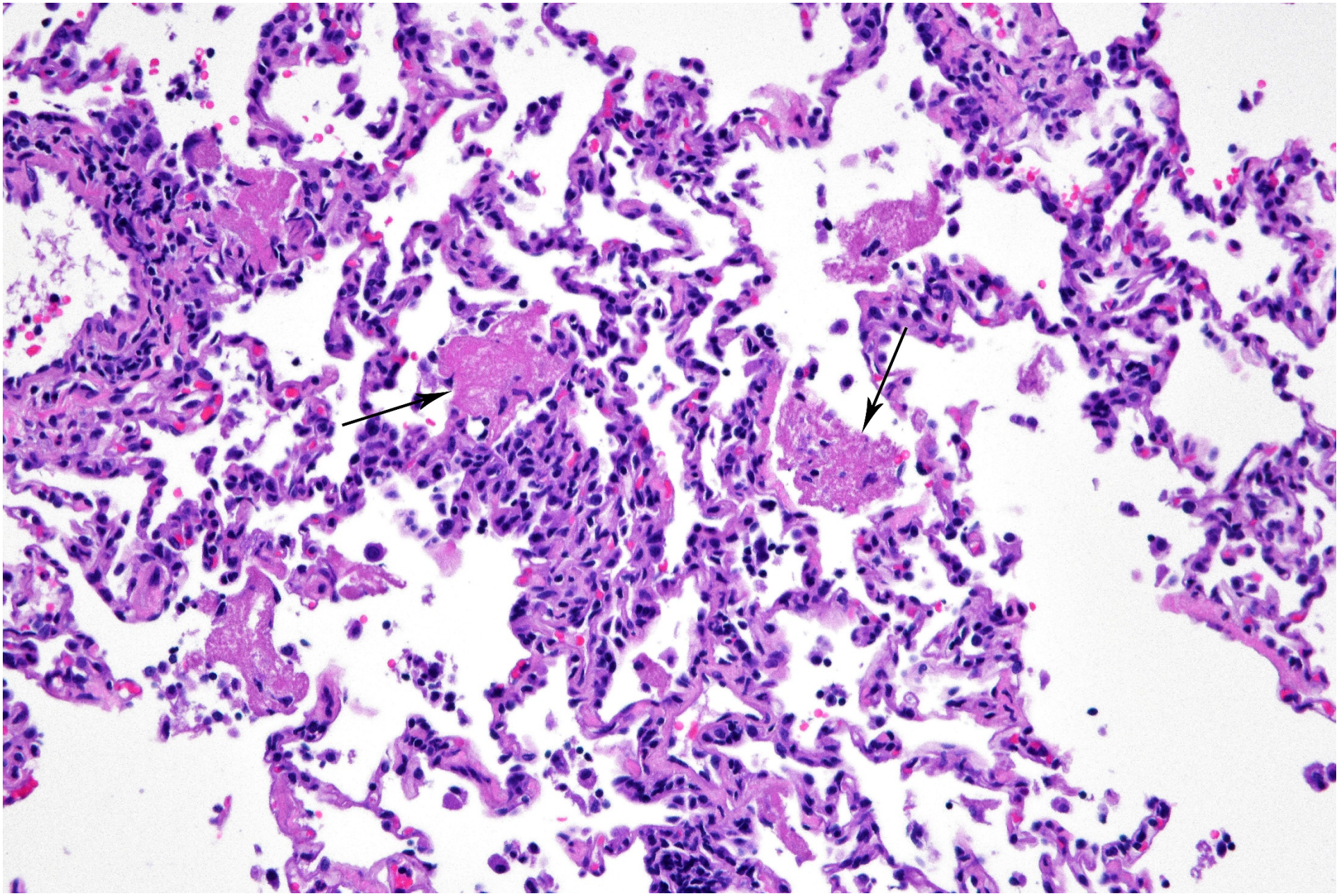


Figure 19

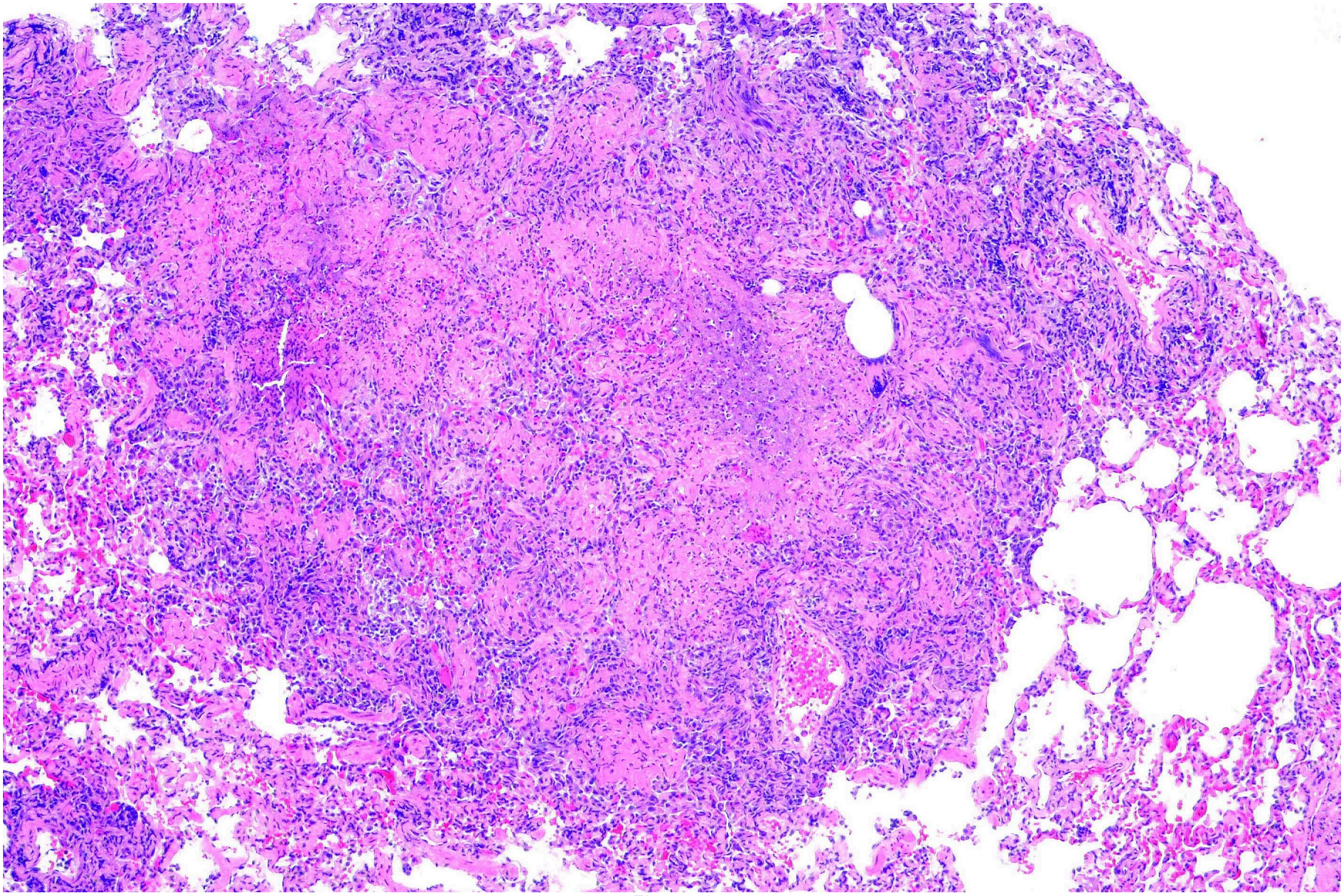


Figure 20

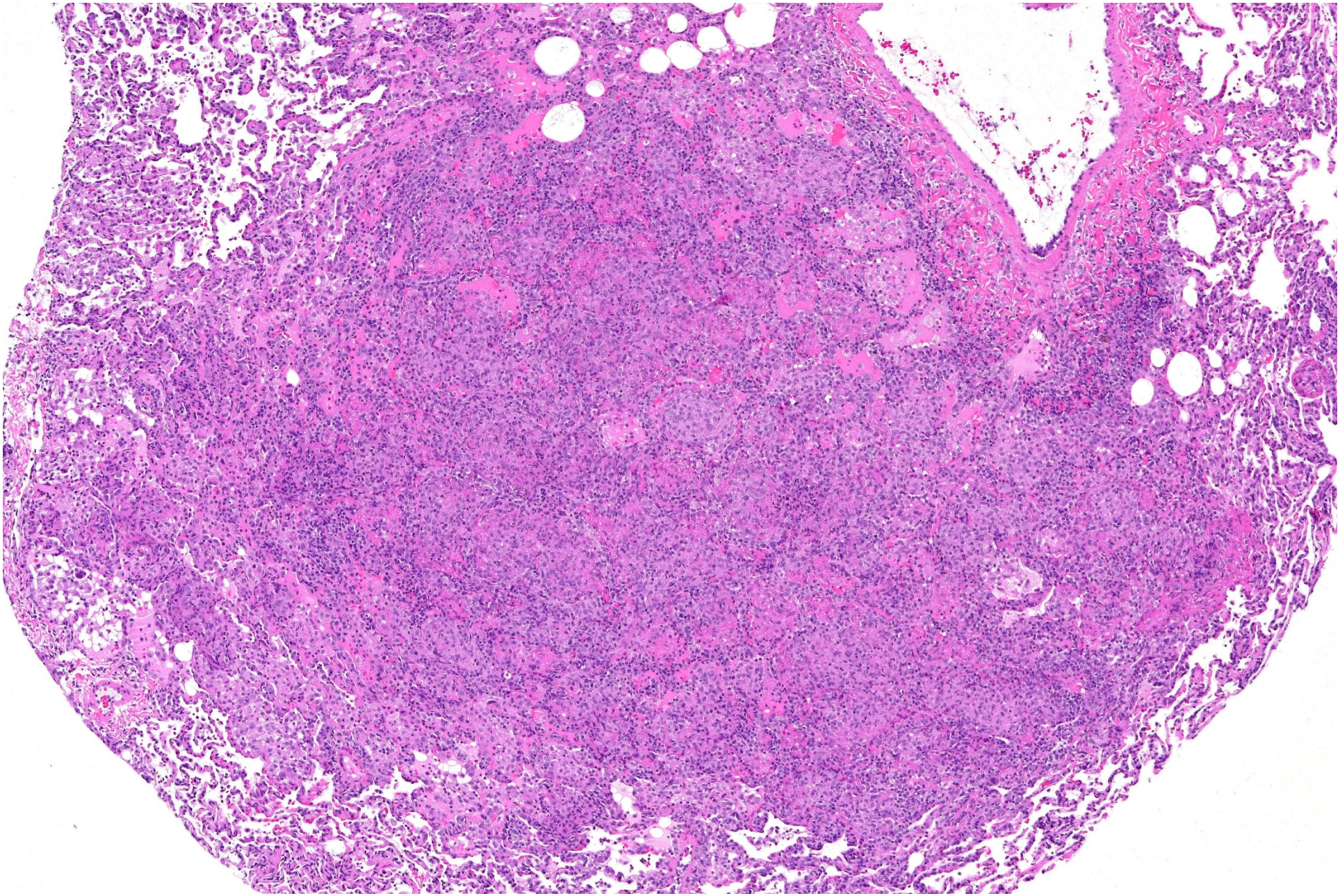


Figure 21

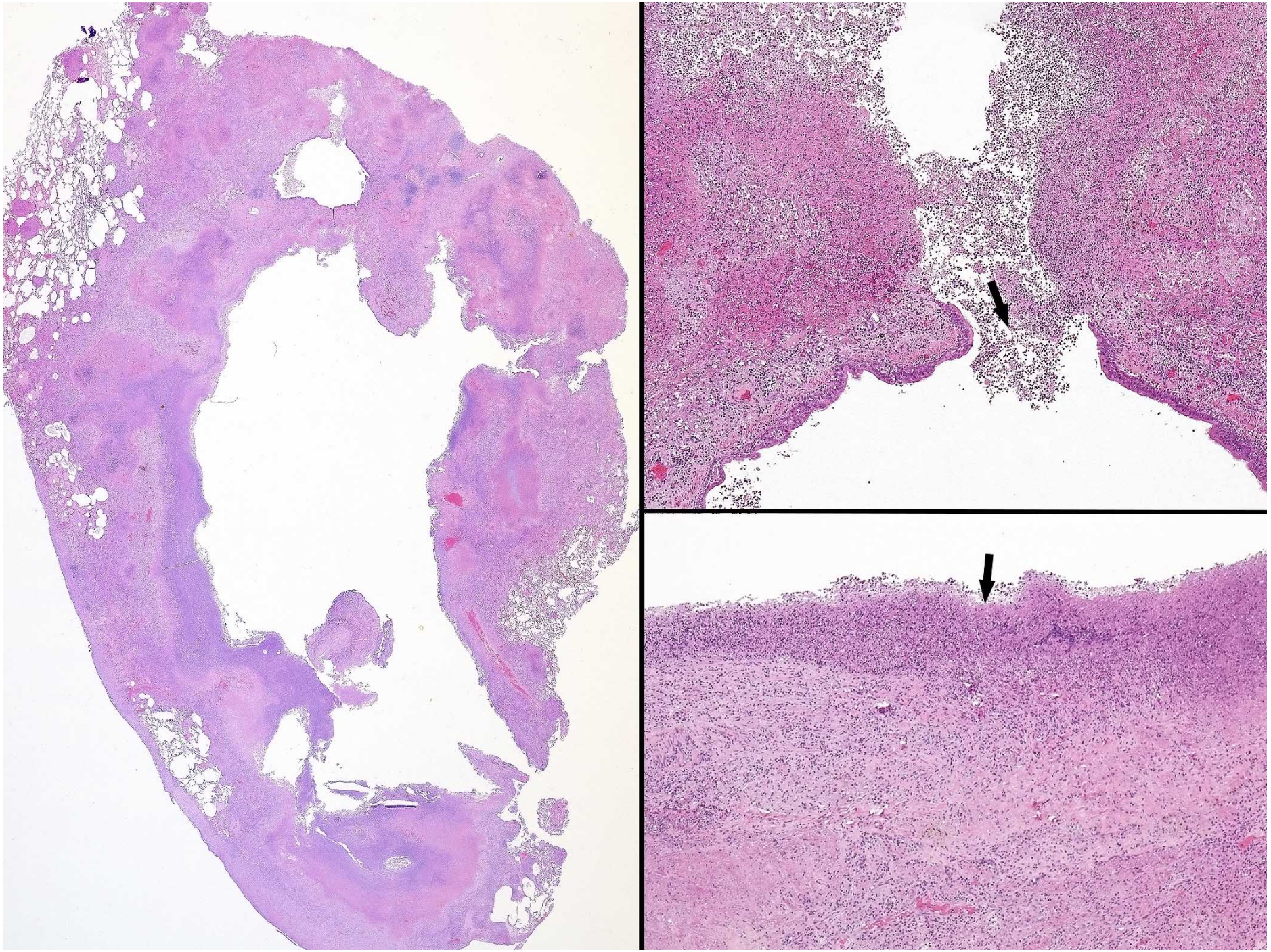


Figure 22

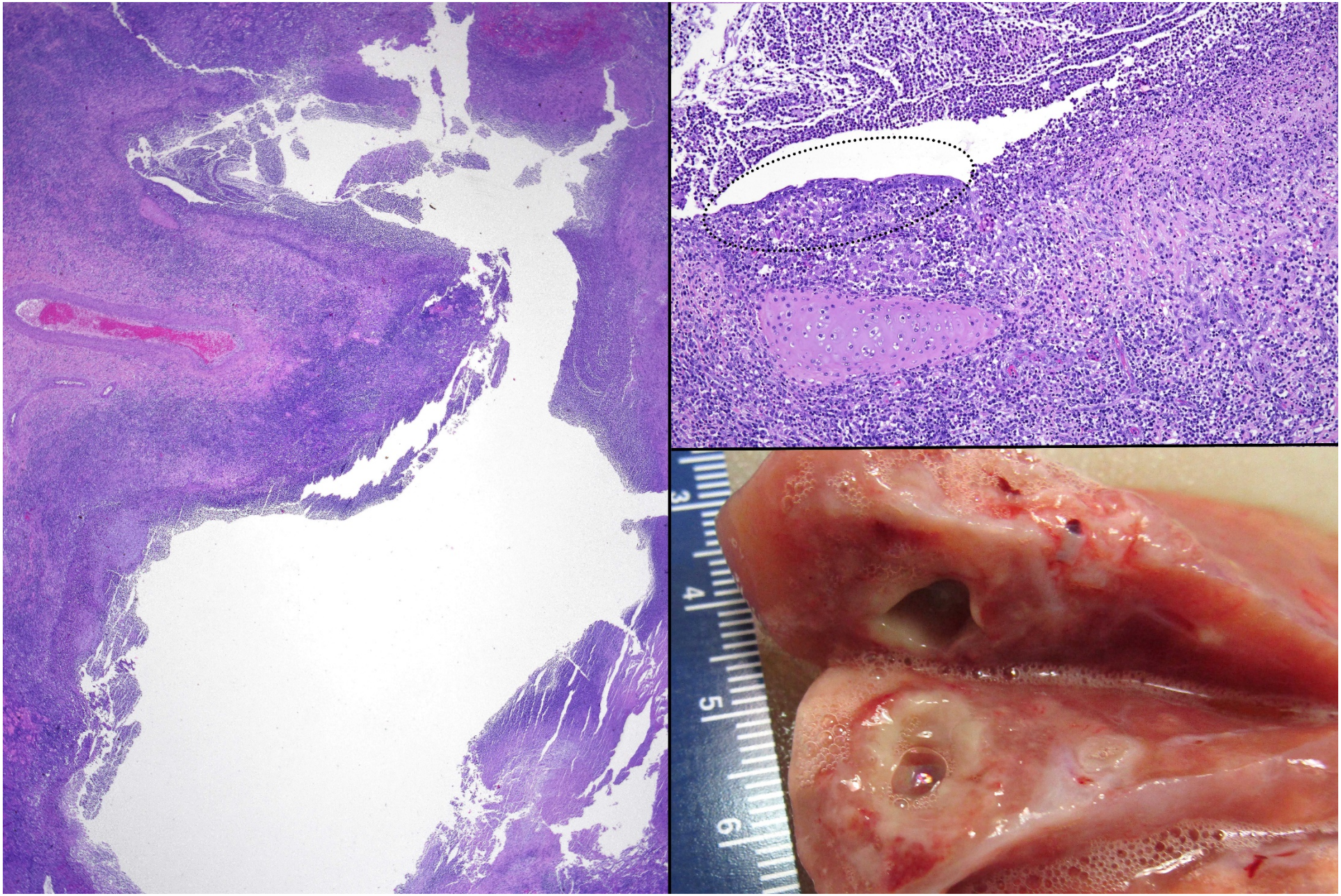


Figure 23