Supplementary Materials for

Spatial interplay patterns of cancer nuclei and tumor-infiltrating lymphocytes (TILs) predict clinical benefit for immune checkpoint inhibitors

Xiangxue Wang et al.

Corresponding author: Xiangxue Wang, xxw345@case.edu; Anant Madabhushi, axm788@case.edu

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Supplementary Material

Image Analysis

We have applied U-Net style network for nuclei segmentation with public training dataset and adversarial training strategy, and tested on our internal dataset with F-score 0.881 (95% CI, 0.873-0.889) on a dataset with 8,000 pathologist annotated nuclei from 100 digitized H&E images. The details our network architecture was shown in supplementary table S3. Then a nested U-Net (Details in table S4) was employed for epithelium and stroma segmentation F-score of 0.853 (95% CI, 0.843-0.862) on a dataset with 100 FOVs of 1000x1000 pixels from 35 patients. Then, the previous reported lymphocyte detection model was applied on our data to generate a F-Score of 0.856 (95% CI, 0.850-0.862) on a dataset of 3,000 lymphocytes from 30 lung cancer images.

Graph Construction

The centroid of each segmented nuclei is treated as vertex V of a graph \( G = (V, E) \), the edge, E, connects any two of vertices is designed to favor the local cell aggregation. In other word, the spatially close cells are more likely to form a systematical function such as lymphocyte infiltration cluster. To formally establish the connection between each pair of individual cells, the spatial distance between pixels is first transformed to a likelihood of aggregation, which is defined as follows:

\[
L(v_i, v_j) = D(v_i, v_j)^{-\alpha},
\]

where \( V_i \) and \( V_j \) are center of any two cells of the same type (since we construct two separate graphs for immune and cancer cells, respectively) and D is the simple Euclidean distance of the center of two cells. The density controlling parameter was empirically set at 0.5 to favor the pairwise connection. The likelihood of cells to be formed within the same clique was determined via an exponentially decaying function of spatial distance, i.e., the physically neighboring cells are more likely to employ/infiltrate together to fulfill immune function. The edge E is then defined as:

\[
E = \{(v_i, v_j) : r < L(v_i, v_j), \forall v_i, v_j \in V\}
\]

where \( r \in [0,1] \) is a distance controlling parameter. We experimented with the value of \( r \) from 0.1 to 0.9 with a step size at 0.05, ultimately we identified 0.45 as yielding the best result for balancing cluster density versus overall efficiency of graph construction.
Fig. S1. Feature distributions among different dataset. Violin plots of feature distributions for HistoTIL composed top features (a-g) and TILs density (h) and ratio (i) across each different dataset.
Fig. S2. Survival analysis of gynecological cancer among ICIs agents. Kaplan-Meier overall survival assessment among ICIs agents (Nivolumab and Pembrolizumab) for patients with gynecological cancer with OS (A, B) and PFS (C, D).
| Index | Feature Name | Description |
|-------|--------------|-------------|
| 1     | epi_nuclei_ZernPol5_Phi | A Zernike polynomial descriptor deformation level 5 (phi) of the shape of the cells within the epithelium tissue |
| 2     | epi_nuclei_ZernPol18_Phi | A Zernike polynomial descriptor deformation level 18 (phi) of the shape of the cells within the epithelium tissue |
| 3     | epi_nuclei_ZernPol19_Phi | A Zernike polynomial descriptor deformation level 19 (phi) of the shape of the cells within the epithelium tissue |
| 4     | contextual_filtareasvarmatK3 | Variance of the area size of cells within a radial region of length 30*K (K=150px at 40X mag.) |
| 5     | stroma_spATIL_intersectedArea | Spatial descriptor: Size of the intersected convex hull areas between the lymphocyte and the non-lymphocyte cells. |
| 6     | spATIL_avg of reciprocal of number of least lym cluster to encompass nuclei cluster | Spatial descriptor: Average of the reciprocal number of the least number of lymphocytes clusters that are surrounding the non-lymphocyte clusters |
| 7     | density_MaxLympGroupingFactor | Density descriptor: The maximum quantity of lymphocyte cluster in a single patch (2000x2000 px at 40X mag.) |
| Feature Label | Contextual (n=87) | Nuclei (n=100) | Spatial TIL (spaTIL) (n=85) | Density TIL (denTIL) (n=19) | General Description |
|---------------|------------------|----------------|---------------------------|-----------------------------|-------------------|
| **Table S2. Feature Names and Categories** | | | | | **Nuclei features involve the morphological quantification of the non-TIL nuclei involving shape, color (intensity) and texture.** |
| **Based on the nuclei features, different quantification metrics are calculated around each nucleus, such as how many, different or similar are compared to the central nuclei. These metrics include, quantity of cells, their shape, area, eccentricity, number of surrounding lymphocytes.** | | | | | **It involves the density of Tumor-infiltrating Lymphocytes involving different specific metrics such as number of TILs per area, ratio of TILs and tumor cells, ratio of TIL cluster over tumor cell cluster.** |
| **it involves the density of Tumor-infiltrating Lymphocytes involving different specific metrics such as number of TILs per area, ratio of TILs and tumor cells, ratio of TIL cluster over tumor cell cluster.** | | | | | **Similar to density, spatial arrangement of TILs is quantified using distinctive graph-based metrics to find niche clusters of TILs surrounding tumor clusters and their number, convex hull shape, proximity is calculated.** |
| Index | Name                                      | Type            | Description                                                                 | Activation Sizes |
|-------|-------------------------------------------|-----------------|-----------------------------------------------------------------------------|------------------|
| 1     | ImageInputLayer                           | Image Input     | 2000x2000x2 images                                                          | 2000 2000 2      |
| 2     | Encoder-Stage-1-Conv-1                    | Convolution     | 64 3x3x2 convolutions with stride [1 1] and padding 'same'                  | 2000 2000 64     |
| 3     | Encoder-Stage-1-ReLU-1                    | ReLU            | ReLU                                                                        | 2000 2000 64     |
| 4     | Encoder-Stage-1-Conv-2                    | Convolution     | 64 3x3x64 convolutions with stride [1 1] and padding 'same'                 | 2000 2000 64     |
| 5     | Encoder-Stage-1-ReLU-2                    | ReLU            | ReLU                                                                        | 2000 2000 64     |
| 6     | Encoder-Stage-1-MaxPool                   | Max Pooling     | 2x2 max pooling with stride [2 2] and padding [0 0 0 0]                      | 1000 1000 64     |
| 7     | Encoder-Stage-2-Conv-1                    | Convolution     | 128 3x3x64 convolutions with stride [1 1] and padding 'same'                | 1000 1000 128    |
| 8     | Encoder-Stage-2-ReLU-1                    | ReLU            | ReLU                                                                        | 1000 1000 128    |
| 9     | Encoder-Stage-2-Conv-2                    | Convolution     | 128 3x3x128 convolutions with stride [1 1] and padding 'same'               | 1000 1000 128    |
| 10    | Encoder-Stage-2-ReLU-2                    | ReLU            | ReLU                                                                        | 1000 1000 128    |
| 11    | Encoder-Stage-2-MaxPool                   | Max Pooling     | 2x2 max pooling with stride [2 2] and padding [0 0 0 0]                      | 500 500 128      |
| 12    | Encoder-Stage-3-Conv-1                    | Convolution     | 256 3x3x128 convolutions with stride [1 1] and padding 'same'               | 500 500 256      |
| 13    | Encoder-Stage-3-ReLU-1                    | ReLU            | ReLU                                                                        | 500 500 256      |
| 14    | Encoder-Stage-3-Conv-2                    | Convolution     | 256 3x3x256 convolutions with stride [1 1] and padding 'same'               | 500 500 256      |
| 15    | Encoder-Stage-3-ReLU-2                    | ReLU            | ReLU                                                                        | 500 500 256      |
| 16    | Encoder-Stage-3-MaxPool                   | Max Pooling     | 2x2 max pooling with stride [2 2] and padding [0 0 0 0]                      | 250 250 256      |
| 17    | Encoder-Stage-4-Conv-1                    | Convolution     | 512 3x3x256 convolutions with stride [1 1] and padding 'same'               | 250 250 512      |
| 18    | Encoder-Stage-4-ReLU-1                    | ReLU            | ReLU                                                                        | 250 250 512      |
| 19    | Encoder-Stage-4-Conv-2                    | Convolution     | 512 3x3x512 convolutions with stride [1 1] and padding 'same'               | 250 250 512      |
| 20    | Encoder-Stage-4-ReLU-2                    | ReLU            | ReLU                                                                        | 250 250 512      |
| 21    | Encoder-Stage-4-DropOut                   | Dropout         | 50% dropout                                                                  | 250 250 512      |
| 22    | Encoder-Stage-4-MaxPool                   | Max Pooling     | 2x2 max pooling with stride [2 2] and padding [0 0 0 0]                      | 125 125 512      |
| 23    | Bridge-Conv-1                             | Convolution     | 1024 3x3x512 convolutions with stride [1 1] and padding 'same'              | 125 125 1024     |
| 24    | Bridge-ReLU-1                             | ReLU            | ReLU                                                                        | 125 125 1024     |
| 25    | Bridge-Conv-2                             | Convolution     | 1024 3x3x1024 convolutions with stride [1 1] and padding 'same'             | 125 125 1024     |
| 26    | Bridge-ReLU-2                             | ReLU            | ReLU                                                                        | 125 125 1024     |
| 27    | Bridge-DropOut                            | Dropout         | 50% dropout                                                                  | 125 125 1024     |
| 28    | Decoder-Stage-1-UpConv                    | Transposed Convolution | 512 2x2x512 transposed convolutions with stride [2 2] and cropping [0 0 0 0] | 250 250 512      |
| 29    | Decoder-Stage-1-UpReLU                    | ReLU            | ReLU                                                                        | 250 250 512      |
| 30    | Decoder-Stage-1-DepthConcatenation        | Depth concatenation | Depth concatenation of 2 inputs                                                | 250 250 1024     |
| 31    | Decoder-Stage-1-Conv-1                    | Convolution     | 512 3x3x1024 convolutions with stride [1 1] and padding 'same'              | 250 250 512      |
| 32    | Decoder-Stage-1-ReLU-1                    | ReLU            | ReLU                                                                        | 250 250 512      |
| 33    | Decoder-Stage-1-Conv-2                    | Convolution     | 512 3x3x512 convolutions with stride [1 1] and padding 'same'               | 250 250 512      |
| 34    | Decoder-Stage-1-ReLU-2                    | ReLU            | ReLU                                                                        | 250 250 512      |
| 35    | Decoder-Stage-2-UpConv                    | Transposed Convolution | 256 2x2x512 transposed convolutions with stride [2 2] and cropping [0 0 0 0] | 500 500 256      |
| 36    | Decoder-Stage-2-UpReLU                    | ReLU            | ReLU                                                                        | 500 500 256      |
| 37    | Decoder-Stage-2-DepthConcatenation        | Depth concatenation | Depth concatenation of 2 inputs                                                | 500 500 512      |
|   | Layer Type            | Filter Size | Stride | Padding | Output Shape |
|---|----------------------|-------------|--------|---------|--------------|
|   | Decoder-Stage-2-Conv-1 | Convolution | 256 3x3x512 | [1 1] | same | 500 500 256 |
|   | Decoder-Stage-2-ReLU-1 | ReLU        |         |         |              | 500 500 256 |
|   | Decoder-Stage-2-Conv-2 | Convolution | 256 3x3x256 | [1 1] | same | 500 500 256 |
|   | Decoder-Stage-2-ReLU-2 | ReLU        |         |         |              | 500 500 256 |
|   | Decoder-Stage-3-UpConv | Transposed Convolution | 128 2x2x256 | [2 2] | [0 0 0 0] | 1000 1000 128 |
|   | Decoder-Stage-3-UpReLU | ReLU        |         |         |              | 1000 1000 128 |
|   | Decoder-Stage-3-DepthConcatenation | Depth concatenation |       |         | Depth concatenation of 2 inputs | 1000 1000 256 |
|   | Decoder-Stage-3-Conv-1 | Convolution | 128 3x3x256 | [1 1] | same | 1000 1000 128 |
|   | Decoder-Stage-3-ReLU-1 | ReLU        |         |         |              | 1000 1000 128 |
|   | Decoder-Stage-3-Conv-2 | Convolution | 128 3x3x128 | [1 1] | same | 1000 1000 128 |
|   | Decoder-Stage-3-ReLU-2 | ReLU        |         |         |              | 1000 1000 128 |
|   | Decoder-Stage-4-UpConv | Transposed Convolution | 64 2x2x128 | [2 2] | [0 0 0 0] | 2000 2000 64 |
|   | Decoder-Stage-4-UpReLU | ReLU        |         |         |              | 2000 2000 64 |
|   | Decoder-Stage-4-DepthConcatenation | Depth concatenation |       |         | Depth concatenation of 2 inputs | 2000 2000 128 |
|   | Decoder-Stage-4-Conv-1 | Convolution | 64 3x3x128 | [1 1] | same | 2000 2000 64 |
|   | Decoder-Stage-4-ReLU-1 | ReLU        |         |         |              | 2000 2000 64 |
|   | Decoder-Stage-4-Conv-2 | Convolution | 64 3x3x64 | [1 1] | same | 2000 2000 64 |
|   | Decoder-Stage-4-ReLU-2 | ReLU        |         |         |              | 2000 2000 64 |
|   | Final-Conv | Convolution | 1 1x1x64 | [1 1] | [0 0 0 0] | 2000 2000 1 |
Table S4. Nested unet network for epithelium-stroma segmentation

| Index | Layer Name       | Activation Shape | Param Number |
|-------|------------------|------------------|--------------|
| 1     | Conv2d-1         | [-1, 32, 512, 512] | 896          |
| 2     | BatchNorm2d-2    | [-1, 32, 512, 512] | 64           |
| 3     | ReLU-3           | [-1, 32, 512, 512] | 0            |
| 4     | Conv2d-4         | [-1, 32, 512, 512] | 9,248        |
| 5     | BatchNorm2d-5    | [-1, 32, 512, 512] | 64           |
| 6     | ReLU-6           | [-1, 32, 512, 512] | 0            |
| 7     | VGGBlock-7       | [-1, 32, 512, 512] | 0            |
| 8     | MaxPool2d-8      | [-1, 32, 256, 256] | 0            |
| 9     | Conv2d-9         | [-1, 64, 256, 256] | 18,496       |
| 10    | BatchNorm2d-10   | [-1, 64, 256, 256] | 128          |
| 11    | ReLU-11          | [-1, 64, 256, 256] | 0            |
| 12    | Conv2d-12        | [-1, 64, 256, 256] | 36,928       |
| 13    | BatchNorm2d-13   | [-1, 64, 256, 256] | 128          |
| 14    | ReLU-14          | [-1, 64, 256, 256] | 0            |
| 15    | VGGBlock-15      | [-1, 64, 256, 256] | 0            |
| 16    | Upsample-16      | [-1, 64, 512, 512] | 0            |
| 17    | Conv2d-17        | [-1, 32, 512, 512] | 27,680       |
| 18    | BatchNorm2d-18   | [-1, 32, 512, 512] | 64           |
| 19    | ReLU-19          | [-1, 32, 512, 512] | 0            |
| 20    | Conv2d-20        | [-1, 32, 512, 512] | 9,248        |
| 21    | BatchNorm2d-21   | [-1, 32, 512, 512] | 64           |
| 22    | ReLU-22          | [-1, 32, 512, 512] | 0            |
| 23    | VGGBlock-23      | [-1, 32, 512, 512] | 0            |
| 24    | MaxPool2d-24     | [-1, 64, 128, 128] | 0            |
| 25    | Conv2d-25        | [-1, 128, 128, 128] | 73,856       |
| 26    | BatchNorm2d-26   | [-1, 128, 128, 128] | 256          |
| 27    | ReLU-27          | [-1, 128, 128, 128] | 0            |
| 28    | Conv2d-28        | [-1, 128, 128, 128] | 147,584      |
| 29    | BatchNorm2d-29   | [-1, 128, 128, 128] | 256          |
| 30    | ReLU-30          | [-1, 128, 128, 128] | 0            |
| 31    | VGGBlock-31      | [-1, 128, 128, 128] | 0            |
| 32    | Upsample-32      | [-1, 128, 256, 256] | 0            |
| 33    | Conv2d-33        | [-1, 64, 256, 256] | 110,656      |
| 34    | BatchNorm2d-34   | [-1, 64, 256, 256] | 128          |
| 35    | ReLU-35          | [-1, 64, 256, 256] | 0            |
|   |   |   |   |
|---|---|---|---|
|36 | Conv2d-36 | [-1, 64, 256, 256] | 36,928 |
|37 | BatchNorm2d-37 | [-1, 64, 256, 256] | 128 |
|38 | ReLU-38 | [-1, 64, 256, 256] | 0 |
|39 | VGGBlock-39 | [-1, 64, 256, 256] | 0 |
|40 | Upsample-40 | [-1, 64, 512, 512] | 0 |
|41 | Conv2d-41 | [-1, 32, 512, 512] | 36,896 |
|42 | BatchNorm2d-42 | [-1, 32, 512, 512] | 64 |
|43 | ReLU-43 | [-1, 32, 512, 512] | 0 |
|44 | Conv2d-44 | [-1, 32, 512, 512] | 9,248 |
|45 | BatchNorm2d-45 | [-1, 32, 512, 512] | 64 |
|46 | ReLU-46 | [-1, 32, 512, 512] | 0 |
|47 | VGGBlock-47 | [-1, 32, 512, 512] | 0 |
|48 | MaxPool2d-48 | [-1, 128, 64, 64] | 0 |
|49 | Conv2d-49 | [-1, 256, 64, 64] | 295,168 |
|50 | BatchNorm2d-50 | [-1, 256, 64, 64] | 512 |
|51 | ReLU-51 | [-1, 256, 64, 64] | 0 |
|52 | Conv2d-52 | [-1, 256, 64, 64] | 590,080 |
|53 | BatchNorm2d-53 | [-1, 256, 64, 64] | 512 |
|54 | ReLU-54 | [-1, 256, 64, 64] | 0 |
|55 | VGGBlock-55 | [-1, 256, 64, 64] | 0 |
|56 | Upsample-56 | [-1, 256, 128, 128] | 0 |
|57 | Conv2d-57 | [-1, 128, 128, 128] | 442,496 |
|58 | BatchNorm2d-58 | [-1, 128, 128, 128] | 256 |
|59 | ReLU-59 | [-1, 128, 128, 128] | 0 |
|60 | Conv2d-60 | [-1, 128, 128, 128] | 147,584 |
|61 | BatchNorm2d-61 | [-1, 128, 128, 128] | 256 |
|62 | ReLU-62 | [-1, 128, 128, 128] | 0 |
|63 | VGGBlock-63 | [-1, 128, 128, 128] | 0 |
|64 | Upsample-64 | [-1, 128, 256, 256] | 0 |
|65 | Conv2d-65 | [-1, 64, 256, 256] | 147,520 |
|66 | BatchNorm2d-66 | [-1, 64, 256, 256] | 128 |
|67 | ReLU-67 | [-1, 64, 256, 256] | 0 |
|68 | Conv2d-68 | [-1, 64, 256, 256] | 36,928 |
|69 | BatchNorm2d-69 | [-1, 64, 256, 256] | 128 |
|70 | ReLU-70 | [-1, 64, 256, 256] | 0 |
|71 | VGGBlock-71 | [-1, 64, 256, 256] | 0 |
|72 | Upsample-72 | [-1, 64, 512, 512] | 0 |
|   | Operation       | Input Shape        | Output Shape |
|---|-----------------|--------------------|--------------|
| 73 | Conv2d-73       | [-1, 32, 512, 512] | 46,112       |
| 74 | BatchNorm2d-74  | [-1, 32, 512, 512] | 64           |
| 75 | ReLU-75         | [-1, 32, 512, 512] | 0            |
| 76 | Conv2d-76       | [-1, 32, 512, 512] | 9,248        |
| 77 | BatchNorm2d-77  | [-1, 32, 512, 512] | 64           |
| 78 | ReLU-78         | [-1, 32, 512, 512] | 0            |
| 79 | VGGBlock-79     | [-1, 32, 512, 512] | 0            |
| 80 | MaxPool2d-80    | [-1, 256, 32, 32]  | 0            |
| 81 | Conv2d-81       | [-1, 512, 32, 32]  | 1,180,160    |
| 82 | BatchNorm2d-82  | [-1, 512, 32, 32]  | 1,024        |
| 83 | ReLU-83         | [-1, 512, 32, 32]  | 0            |
| 84 | Conv2d-84       | [-1, 512, 32, 32]  | 2,359,808    |
| 85 | BatchNorm2d-85  | [-1, 512, 32, 32]  | 1,024        |
| 86 | ReLU-86         | [-1, 512, 32, 32]  | 0            |
| 87 | VGGBlock-87     | [-1, 512, 32, 32]  | 0            |
| 88 | Upsample-88     | [-1, 512, 64, 64]  | 0            |
| 89 | Conv2d-89       | [-1, 256, 64, 64]  | 1,769,728    |
| 90 | BatchNorm2d-90  | [-1, 256, 64, 64]  | 512          |
| 91 | ReLU-91         | [-1, 256, 64, 64]  | 0            |
| 92 | Conv2d-92       | [-1, 256, 64, 64]  | 590,800      |
| 93 | BatchNorm2d-93  | [-1, 256, 64, 64]  | 512          |
| 94 | ReLU-94         | [-1, 256, 64, 64]  | 0            |
| 95 | VGGBlock-95     | [-1, 256, 64, 64]  | 0            |
| 96 | Upsample-96     | [-1, 256, 128, 128]| 0            |
| 97 | Conv2d-97       | [-1, 128, 128, 128]| 589,952      |
| 98 | BatchNorm2d-98  | [-1, 128, 128, 128]| 256          |
| 99 | ReLU-99         | [-1, 128, 128, 128]| 0            |
| 100| Conv2d-100      | [-1, 128, 128, 128]| 147,584      |
| 101| BatchNorm2d-101 | [-1, 128, 128, 128]| 256          |
| 102| ReLU-102        | [-1, 128, 128, 128]| 0            |
| 103| VGGBlock-103    | [-1, 128, 128, 128]| 0            |
| 104| Upsample-104    | [-1, 128, 256, 256]| 0            |
| 105| Conv2d-105      | [-1, 64, 256, 256]| 184,384      |
| 106| BatchNorm2d-106 | [-1, 64, 256, 256]| 128          |
| 107| ReLU-107        | [-1, 64, 256, 256]| 0            |
| 108| Conv2d-108      | [-1, 64, 256, 256]| 36,928       |
| 109| BatchNorm2d-109 | [-1, 64, 256, 256]| 128          |
|   | Layer       | Input Shape     | Output Shape | Params |
|---|-------------|-----------------|--------------|--------|
| 110 | ReLU-110    | [-1, 64, 256, 256] |              | 0      |
| 111 | VGGBlock-111| [-1, 64, 256, 256] |              | 0      |
| 112 | Upsample-112| [-1, 64, 512, 512] |              | 0      |
| 113 | Conv2d-113  | [-1, 32, 512, 512] |              | 55328  |
| 114 | BatchNorm2d-114 | [-1, 32, 512, 512] |              | 64     |
| 115 | ReLU-115    | [-1, 32, 512, 512] |              | 0      |
| 116 | Conv2d-116  | [-1, 32, 512, 512] |              | 9248   |
| 117 | BatchNorm2d-117 | [-1, 32, 512, 512] |              | 64     |
| 118 | ReLU-118    | [-1, 32, 512, 512] |              | 0      |
| 119 | VGGBlock-119| [-1, 32, 512, 512] |              | 0      |
| 120 | Conv2d-120  | [-1, 1, 512, 512]  |              | 33     |
Code and Data

All data for training and validation the detection and segmentation models are available from https://doi.org/10.5281/zenodo.6415129. Access to original whole slide images from the University of Pennsylvania, Cleveland Clinic Foundation, Yale University, and University Hospital Cleveland Medical Center (used with permission for this study) could be requested directly from these institutions via their data access request forms. Subject to the institutional review boards' ethical approval, deidentified data can be made available as a validation subset.
| Section & Topic | No | Item | Reported on page |
|-----------------|----|------|-----------------|
| **TITLE OR ABSTRACT** | | | |
| 1 | Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC) | 2 |
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| 3 | Scientific and clinical background, including the intended use and clinical role of the index test | 3 |
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| **Participants** | | | |
| 6 | Eligibility criteria | 10 |
| 7 | On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry) | 10 |
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| 13b | Whether clinical information and index test results were available to the assessors of the reference standard | 12-13 |
| **Analysis** | | | |
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| 15 | How indeterminate index test or reference standard results were handled | 13-14 |
| 16 | How missing data on the index test and reference standard were handled | 11-13 |
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| 29 | Where the full study protocol can be accessed | 10 |
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