

Papanicolaou (PAP) analysis of sputum samples

Methodology

Three PAP-stained slides for each of the 69 participants in the high-risk cohort who had shown a normal CT scan and 16 participants in the cancer cohort (both part of the clinical trial NCT00894127) were read independently by two individuals (read 1 and read 2). Each slide was classified into one of 10 categories (see below) based on cellular criteria including nuclear/cytoplasm ratio, chromatin content, cellular cohesion, architectural features, and counts of squamous, glandular, stromal, and hematopoietic cells, following a standardized protocol. The classification categories were:

0. No significant epithelial abnormalities
1. Regular metaplasia
2. Mild atypia
3. Moderate atypia
4. Severe (marked) atypia
5. Carcinoma in situ
6. Invasive carcinoma
7. Squamous cell carcinoma
8. Adenocarcinoma
9. Large cell carcinoma
10. Small cell carcinoma

Additionally, to assess "closeness" of reader classifications, the original classifications were further grouped into three summary categories:

1. No abnormality / regular metaplasia (original codes 0-1)
2. Atypia (original codes 2 - 4)
3. Cancer (original codes 5 - 10)

Results

Of the 69 high-risk subjects, four were classified with cancer by one (three cases) or both readers (one case). In each of the four subjects, evidence of cancer was found on a single slide and not found on the other two (**Table 1**, cases highlighted in yellow). Only two of the 16 cancer subjects were classified with cancer by both readers. Both cases were identified independently on slides 2 and 3, with no cancer classification on slide 1 (**Table 2**, cases highlighted in yellow). For one additional case, cancer cells were identified by one reader on a single slide (**Table 2**, case highlighted in orange).

The agreement between read 1 and read 2 on the original classification (0-10) for high-risk subjects ranged between 78.3% (slide 2) and 85.5% (slides 1 and 3). The agreement on the summary categories (no abnormality, atypia, cancer) ranged between 94.2% (Slide 2) and 97.1% (Slide 1). The agreement between read 1 and read 2 on the original classification (0-10) for cancer subjects ranged between 62.5% (Slide 3) and 87.5% (Slide 1). The agreement on the summary categories (no abnormality, atypia, cancer) ranged between 87.5% (slide 3) and 100.0% (slides 1 and 2).

After the readings from PAP-stained slides were evaluated in more than half of the cancer subjects (n=16), we found that PAP could detect cancer cells in only 18.75% of the cases. With such poor sensitivity to detect true positives, it was decided to discontinue the full PAP analysis and limit it to one slide for the purpose of determining the presence of macrophages.

Table 1. Results of PAP analysis on 69 participants in the high-risk cohort

Part. ID #	Slide A		Slide B		Slide C	
	Read 1	Read 2	Read 1	Read 2	Read 1	Read 2
2.	No Abnormalities	No Abnormalities	Mild	Mild	Mild	Regular Metaplasia
4.	Mild	Mild	Moderate	Mild	Mild	Mild
6.	Moderate	Moderate	Moderate	Mild	Moderate	Moderate
7.	Mild	Mild	Mild	Mild	Mild	Mild
9.	Moderate	Moderate	Moderate	Mild	Mild	Moderate
10.	Mild	Mild	Mild	Mild	Mild	Mild
11.	Mild	Mild	Mild	Mild	Mild	Mild
12.	Mild	Mild	Mild	Mild	Moderate	Moderate
14.	Mild	Mild	Moderate	Moderate	Moderate	Moderate
16.	Mild	Mild	Mild	Mild	Mild	Mild
17.	Mild	Mild	Mild	Mild	Mild	Mild
18.	Mild	Mild	Mild	Mild	Severe	Severe
20.	No Abnormalities	No Abnormalities	Severe	Severe	No Abnormalities	No Abnormalities
22.	Mild	Mild	Mild	Mild	Moderate	Moderate
31.	Moderate	Moderate	Moderate	Moderate	Mild	Mild
32.	Mild	Mild	Mild	Moderate	Severe	Severe
33.	Moderate	Moderate	Moderate	Mild	Moderate	Moderate
38.	Mild	Mild	Moderate	Mild	Moderate	Mild
40.	Moderate	Moderate	Mild	Mild	Moderate	Moderate
44.	Mild	Mild	Moderate	Mild	Mild	Mild

45.	Mild	No Abnormalities	No Abnormalities	No Abnormalities	Mild	Mild
50.	Moderate	Moderate	Mild	Mild	Moderate	Moderate
55.	Mild	Mild	Mild	Mild	Mild	Mild
57.	Mild	Mild	No Abnormalities	No Abnormalities	Mild	Mild
60.	No Abnormalities	Mild	Mild	No Abnormalities	Moderate	Moderate
64.	Mild	Mild	Moderate	Mild	Mild	Mild
65.	Moderate	Moderate	Mild	Mild	Moderate	Moderate
66.	Moderate	Moderate	Moderate	Moderate	Mild	Mild
68.	Mild	Mild	Mild	Mild	Mild	Mild
69.	Mild	Mild	Mild	Moderate	Moderate	Moderate
70.	Mild	Moderate	Mild	Mild	Carcinoma in Situ	Severe
72.	Moderate	Mild	Moderate	Moderate	Severe	Severe
74.	Moderate	Moderate	Moderate	Mild	Moderate	Moderate
77.	Moderate	Mild	Mild	Mild	Moderate	Mild
78.	Mild	Mild	Mild	Mild	Mild	Mild
80.	Mild	Mild	Mild	Mild	Mild	Mild
81.	Moderate	Moderate	Severe	Severe	Moderate	Moderate
82.	Moderate	Moderate	Mild	Mild	Moderate	Moderate
84.	Mild	Mild	Mild	Mild	Mild	Mild
87.	Moderate	Moderate	Moderate	Moderate	Mild	Mild
88.	Mild	Mild	Moderate	Moderate	Moderate	Moderate
90.	Moderate	Mild	Mild	Small Cell Carcinoma	Mild	Mild
91.	Moderate	Moderate	Mild	Mild	Mild	Mild
92.	Moderate	Mild	Moderate	Moderate	Mild	Mild
93.	Mild	Mild	Moderate	Moderate	Severe	Moderate
97.	Mild	Mild	Mild	Mild	Mild	Mild
98.	Mild	Mild	Severe/ Squamous Cell Carcinoma	Squamous Cell Carcinoma	Moderate	Moderate
100.	Regular Metaplasia	Regular Metaplasia	No Abnormalities	No Abnormalities	Mild	Mild
102.	Mild	Mild	Mild	Mild	Moderate	Moderate
104.	Moderate	Moderate	Mild	Mild	Moderate	Severe
105.	Mild	Mild	No Abnormalities	No Abnormalities	Mild	Mild

106.	Mild	Mild	Mild	Moderate	Mild	Mild
108.	Moderate	Mild	Mild	Mild	Mild	Mild
109.	Mild	Mild	Mild	No Abnormalities	No Abnormalities	Mild
110.	Mild	Mild	Mild	Mild	Mild	Mild
113.	Moderate	Mild	Moderate	Moderate	Mild	Mild
114.	Mild	Mild	Mild	Mild	Mild	Mild
115.	Mild	Mild	Mild	Mild	Moderate	Mild
117.	Mild	Mild	No Abnormalities	No Abnormalities	Mild	Mild
122.	Mild	Mild	Moderate	Moderate	Mild	Mild
123.	Moderate	Moderate	Mild	Mild	Mild	Mild
124.	Mild	Moderate	Moderate/ Severe	Squamous Cell Carcinoma	Mild	Mild
126.	Mild	Mild	Mild	Mild	Mild	Moderate
127.	Moderate	Moderate	Mild	Mild	Mild	Mild
128.	Severe	Severe	Moderate	Moderate	Moderate	Moderate
129.	Mild	Mild	No Abnormalities	No Abnormalities	Mild	Mild
130.	Moderate	Moderate	Mild	Mild	Mild	Mild
131.	Severe	Severe	Mild	Mild	Moderate	Moderate
132.	Mild	Mild	Mild	Mild	Mild	Mild

Table 2. Results of PAP analysis on 16 participants in the cancer cohort

Part. ID #	Slide A		Slide B		Slide C	
	Read 1	Read 2	Read 1	Read 2	Read 1	Read 2
134.	Severe	Severe	Severe/ Squamous Cell Carcinoma	Moderate	Mild	Mild
136.	Mild	Mild	Mild	Moderate	Mild	Mild
138.	Mild	Mild	Severe	Severe	Mild	Mild
139.	Mild	Mild	Mild	Mild	Moderate	Mild
143.	Severe	Moderate	Moderate	Moderate	Moderate	Mild
144.	Mild	Mild	Severe	Severe	Mild	Mild
147.	Mild	Mild	Mild	Mild	Mild	No Abnormalities
148.	Mild	Mild	Mild	Mild	Mild	Mild
149.	Mild	Mild	Mild	Mild	Mild	Mild
155.	Mild	Mild	Moderate	Moderate	Moderate	Moderate

156.	Mild	Mild	Mild	Mild	No Abnormalities	No Abnormalities
157.	Moderate	Moderate	Moderate	Moderate	Severe	Moderate
158.	Mild	Mild	Adeno-carcinoma	Adeno-carcinoma	Carcinoma in Situ/ Squamous Cell Carcinoma	Moderate
160.	Moderate	Moderate	Mild	Mild	Moderate	Mild
161.	Mild	Mild	Mild	Moderate	Mild	Mild
167.	Severe	Mild	Severe/ Squamous Cell Carcinoma	Squamous Cell Carcinoma	Squamous Cell Carcinoma	Squamous Cell Carcinoma