Clinical Policy Bulletin: 
Flow Cytometry, DNA Ploidy, and S-phase Fraction

Number: 0351

Policy

I. Aetna considers flow cytometry for cell surface markers medically necessary for any of the following conditions:

A. Abnormal tissue, bone marrow, or blood histology when the results are suspicious for lymphoma, leukemia, or myelodysplastic syndrome and where the physician must distinguish reactive from neoplastic conditions; or
B. B-cell monitoring for immunosuppressive disorders; or
C. Diagnosis of Sezary syndrome; or
D. Hairy cell monitoring; or
E. Hereditary persistence of fetal hemoglobin (HPFH), in persons with unexplained increases in hemoglobin F; or
F. Hereditary spherocytosis, in persons with Coombs’ negative hemolytic anemia; or
G. Immunophenotyping for leukemia, lymphoma, or myelodysplastic syndrome; or
H. Mast cell disease; or
I. Measurement of CD4/CD8 ratio from bronchiolar lavage fluid for diagnosis of sarcoidosis; or
J. Multiple myeloma; or
K. Paroxysmal nocturnal hemoglobinuria; or
L. Post-operative monitoring of members who have undergone organ transplantation; or
M. Primary immunodeficiencies; or
N. T-cell monitoring for HIV infection and AIDS.

II. A National Institutes of Health consensus development conference concluded that measurement of flow cytometry-derived DNA content
Flow cytometry is an emerging technique, which involves the separation, classification and quantitation of cell types by: (i) cell surface antigens (phenotype); (ii) DNA content (ploidy) (DNA index); and (iii) DNA flow cytometric proliferation analysis (S-phase fraction or % S-phase). The basic technique involves passage of a monocellular stream of cells through a beam of laser light after cell surface antigens have been tagged with fluorescent monoclonal antibodies; complex computerized instruments are then used to sort normal from abnormal cells and also subgroups of the same cell type. Data are most often collected as a bar-graph histogram, which is then displayed visually as a densitometer tracing of the bar graph; the concentration of cells in each bar appears as a separate peak for each cell category, with a peak height proportional to the number of cells in each bar of the bar graph. For example, lymphocytes can be separated into B- and T-cell categories; the T-cells can be further phenotyped as helper/inducer, suppressor/cytotoxic, or natural killer cell types.
Using fluorescent dye that stains nucleic acids, flow cytometry methods have also been applied to measure nuclear deoxyribonucleic acid (DNA) content (ploidy) as a prognostic indicator of solid tumors based on the fact that malignant cells sometimes show abnormalities in total chromosome number and the frequency of these abnormalities generally increases with progression to higher-grade tumors. In such testing, DNA diploid tumors are those in which a single peak containing an amount of DNA similar to normal control cells is generated by flow cytometry. DNA aneuploid tumors have additional peaks on DNA histogram, presumably representing cells containing more or less nucleic acid than is found in 46 normal chromosomes. A more quantitative method of expression is the DNA index (DI), which is the ratio of the mean tumor sample G0/G1 DNA content of normal diploid reference cells. The greater the deviation of the DI from 1, the more "aneuploid" the tumor.

Another type of measurement for DNA is the assessment of % S-phase or the S-phase fraction (SPF), the percentage or proportion of cells preparing for mitosis by their active doubling of DNA. Since tumor cells tend to replicate more readily than normal cells, increased SPF activity can therefore raise the question of malignancy. In many tumors, a high SPF correlates positively with poor differentiation, increasing tumor size, and degree of aggressiveness in tumor spread, which all have prognostic significance.

However, controversy exists concerning the use of DNA content (aneuploid versus diploid status) as an independent prognostic indicator. Basic and clinical studies have reached different conclusions concerning its value. While many of the earlier studies reported that diploid carcinomas had significantly or considerably better prognosis than aneuploid ones, some more recent studies do not confirm this or do not find that ploidy is a significant independent risk factor. The results of studies on DNA content in different types of tumors have yielded varying results. Aneuploidy, which is thought to be most often associated with malignancy, has been shown to occur in some non-neoplastic cell populations as part of the reaction to or regeneration after inflammation or tissue destruction and has also been reported in some benign tumors. While groups of diploid patients have better prognoses than groups of non-diploid patients, ploidy status may have uncertain prognostic value in individual patients. A small biopsy demonstrating diploid tumor may be missing a significant underlying non-diploid component. Controversy remains as to the nature of the relationship between histologic grade and tumor stage with the degree of aneuploidy. And, lastly, standards for tissue preparation and analysis to insure reproducibility are not yet established from lab to lab.

Increased S-phase activity is somewhat better accepted as an unfavorable prognostic sign. Unfortunately, SPF is technically more difficult to measure accurately and its usefulness is limited by the lack of mutually accepted technical standards and methodologies between laboratories, including a uniform procedure for assaying cancers with multiple subpopulations of different kinetics. Not all tumors with increased S-phase fraction are malignant; not all malignant tumors with increased S-phase fraction metastasize; and not all malignant tumors with relatively small S-phase fraction fail to metastasize. Moreover, the S-phase peak is usually not large, even when considerable S-activity is occurring. In DNA
aneuploid tumors, the multiple peaks each represent a different cellular population; however, proliferation results are generally reported from only one peak, complicating the interpretation of the results. Another problem of definition is that of high versus low S phase; these are frequently defined retrospectively or arbitrarily as being above or below the median for all samples assayed. Such post hoc definitions may introduce bias and should in the future be replaced by laboratory-specific, prospectively determined, biologically relevant cutoffs.

The American Society of Clinical Oncology (2001) prepared evidence-based guidelines on the use of tumor markers in breast cancer and colorectal cancer, and reached the following conclusions. Regarding DNA ploidy or flow cytometric proliferation analysis as a marker for colorectal cancer, ASCO concluded that "[p]resent data are insufficient to recommend DNA flow cytometrically-derived ploidy (DNA index) for the management of colorectal cancer." Regarding the use of DNA flow cytometrically derived parameters as markers for breast cancer, ASCO concluded that: a) "[p]resent data are insufficient to recommend obtaining DNA flow cytometry-derived estimates of DNA content or S-phase in breast tissue"; and b) "DNA flow cytometry-derived ploidy are not recommended to be used to assign a patient to prognostic groupings. There is insufficient evidence to recommend the use of S-phase determination for assigning patients to prognostic groupings."

In conclusion, DNA flow cytometry-derived estimates of DNA ploidy and S-phase proliferation have been correlated with cancer patient outcome in many studies. However, the evidence is almost entirely from retrospective studies with multiple cut-off points for defining high- and low-risk populations. The final value and role for DNA ploidy and S-phase fraction testing in current clinical practice remains to be finally determined. The data are contradictory (hence the present controversy between the NIH and HCFA’s coverage policies) as to whether the prognostic information is independent of other recognized predictors, particularly stage, and further evaluation is required to determine if these techniques will provide useful prognostic information regarding individual patient survival, health outcome, and response to therapy.

Habermehl et al (2004) examined flow-cytometric DNA values of pediatric intracranial tumors and attempted to establish DNA analysis as a potential prognostic parameter. A total of 29 brain tumor specimens from 26 pediatric patients were cryo-preserved within a 3-year period. The DNA content was measured by flow cytometry. Six of the tumor specimens had aneuploid DNA patterns. The median of the proliferation index was lower in the survivor group compared with the non-survivor group (36.4 and 47.5 %, respectively). Ten of the 26 patients are still alive, 8 were lost to follow-up, and 8 died. Flow-cytometric DNA analysis may be a helpful tool for examining brain tumors in children. The small size of this study could not establish flow cytometry as a definite prognostic factor. Further prospective multi-center studies are needed to assess the prognostic significance of flow-cytometric DNA analysis.

Gastric cancer is still a common cause of cancer-related deaths worldwide, despite improved diagnostic and therapeutic implications. Hence, early diagnosis has critical importance. Flow cytometry reveals rapid and reproducible quantification of nuclear DNA content of disaggregated tissues and assessment of its significance in various malignant and pre-cancerous lesions. In a multi-center
Yasa and colleagues (2005) examined 121 patients with gastric cancer, chronic atrophic gastritis, gastric polyps, intestinal metaplasia, and gastric dysplasia as well as 36 healthy controls. Flow cytometric measurements of DNA ploidy, total S-phase, G2M-phase and proliferative indexes were analyzed on fresh gastric biopsy specimens obtained by gastroscopy. DNA aneuploidy was present in 43.8% of the gastric cancers (p < 0.05). These investigators found a DNA aneuploidy rate of 15.4% in chronic atrophic gastritis, 15.4% in intestinal metaplasia and 25% in epithelial dysplasia. One of 9 polyps had aneuploidy. None of the normal gastric mucosa samples showed aneuploidy. The controls had lower rates of total S-phase and proliferative indexes (p < 0.05). The authors concluded that DNA flow cytometry may be offered as an objective diagnostic tool for early detection of malignant transformation in gastric lesions. Moreover, Russo et al (2001) who stated that DNA ploidy and SPF, when associated with clinico-pathological staging, might be useful for the identification of gastric cancer patients who have different risks for death or relapse of disease. In addition, Jiao et al (2004) noted that SPF may be a more useful indicator of aggressive behavior in gastric cancers than DNA aneuploidy.

The American Society of Clinical Oncology's update of recommendations for the use of tumor markers in breast cancer (Harris et al, 2007) noted that there is insufficient evidence to support routine use in clinical practice of DNA/ploidy by flow cytometry. ASCO's updated recommendations on the use of tumor markers in colorectal cancer state that neither flow-cytometrically derived DNA ploidy nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer (Lockr et al, 2006).

Wolfson et al (2008) examined possible associations between measurements of DI, SPF, and tumor heterogeneity (TH) using flow cytometry and overall survival (OS) for patients with invasive cervical carcinoma treated with definitive irradiation. A total of 57 patients with International Federation of Obstetrics and Gynecology Stages IB(2) through IVB cervical carcinomas treated with definitive radiotherapy with or without concurrent chemotherapy were enrolled into this registry study that involved flow cytometric analysis of fresh tissue from each cervical cancer obtained by pre-treatment biopsy. These specimens were evaluated for DNA aneuploidy (DI less than or equal to 1.5 versus greater than 1.5), SPF (less than or equal to 15% versus greater than 15%), and TH (uniploid versus multiploid). In these analyses, 27 of the patients were treated in Radiation Therapy Oncology Group protocol 9001, and an additional 30 were offered chemoradiation at a single institution. Forty-one patients had DI less than or equal to 1.5 and 16 DI greater than 1.5. Twenty-nine patients had SPF less than or equal to 15%, 26 greater than 15%, and 2 had no determinable SPF. Forty-three patients had uniploid and 14 multiploid tumors. The 4-year estimated OS rate for the entire study cohort was 62% (95% confidence interval: 48% to 74%). With a median follow-up of 3.7 years, there were no observable associations by univariate analysis for DI, SPF, or TH concerning patient survival. The authors concluded that there were no statistically significant associations among DI, SPF, or TH and patient outcome. They stated that additional studies are needed to identify tumor biomarkers that could predict patients at risk for disseminated disease. Furthermore, Dabic et al (2008) stated that clinical stage and architectural grade are significant predictors for survival of patients with cervical
adenocarcinoma. Status of HPV infection, flow cytometric parameters, nuclear grade and menstrual status do not predict patient survival.

Suehiro and colleagues (2008) stated that many investigators have reported that aneuploidy detected by flow cytometry is a useful prognostic marker in patients with endometrial cancer. Laser scanning cytometry (LSC) is a technology similar to flow cytometry but is more feasible for clinical laboratory use. These investigators evaluated the usefulness of DNA ploidy detected by LSC as a prognostic marker in patients with endometrial cancer and examined genetic and epigenetic factors related to aneuploidy. Endometrial cancer specimens from 106 patients were evaluated. The methylation status of CDH13, Rassf1, SFRP1, SFRP2, SFRP4, SFRP5, p16, hMLH1, MGMT, APC, ATM, and WIF1 and mutations in the p53 and CDC4 genes were investigated. Laser scanning cytometry was performed to determine DNA ploidy. Fluorescence in situ hybridization was done with chromosome-specific centromeric probes to assess chromosomal instability. Uni-variate and multi-variate analyses revealed that p53 mutation and lack of CDH13 hypermethylation associated positively with aneuploidy. Uni-variate analysis showed that aneuploidy, chromosomal instability, and lack of CDH13 hypermethylation as well as surgical stage were significantly predictive of death from endometrial cancer. Furthermore, multi-variate analysis revealed that stage in combination with either DNA aneuploidy or lack of CDH13 hypermethylation was an independent prognostic factor. The authors concluded that these findings suggested that analysis of DNA ploidy and methylation status of CDH13 may help predict clinical outcome in patients with endometrial cancer. Moreover, they stated that prospective randomized trials are needed to confirm the validity of an individualized approach, including determination of tumor ploidy and methylation status of CDH13, to management of endometrial cancer patients.

Ludovini et al (2008) assessed the relationship between a panel of biological markers (p53, Bcl-2, HER-2, Ki67, DNA ploidy and S-phase fraction) and clinical-pathological parameters and its impact on outcome in non-small cell lung cancer (NSCLC). Tumor tissue specimens obtained following surgical resection were collected from consecutive patients with NSCLC. These researchers used an immunocytochemical technique for p53, Bcl-2, HER-2 and Ki67 analysis in fine-needle aspirates obtained from surgical samples that were also evaluated by flow cytometric DNA analysis using a FACScan flow cytometer. From April 2000 to December 2005, a total of 136 patients with radically resected NSCLC were recruited. Median age was 66 years (range of 31 to 84 years), male/female ratio 117/19, ECOG performance status 0/1 127/4, stage I/II/III 76/25/35, squamous/adenocarcinoma/large-cell/mixed histology 62/49/17/8, smokers yes/no 121/11. Positivity of p53, Bcl-2, HER-2 and Ki67 was detected in 51.4 %, 27.9 %, 25.0 % and 55.8 % of the samples, respectively; 82.9 % of the cases revealed aneuploid DNA histograms and 56.7 % presented an S-phase fraction of more than 12 %. Statistically significant associations between high Ki67 and poorly differentiated tumors (p = 0.016) and a smoking history (p = 0.053); p53 positivity and high Ki67 (p = 0.002); HER-2 positivity and adenocarcinoma subtype (p = 0.015) and presence of lymph node involvement (p = 0.006); and Bcl-2 positivity and squamous cell carcinoma subtype (p = 0.058) were observed. At uni-variate analysis, high Ki67 proved to be the only marker associated with disease-free survival (p = 0.047). After adjusting for stage, none of the examined immunocytochemical markers emerged as an independent factor for disease-free
and overall survival; only pathological stage was identified as an independent prognostic factor for disease-free survival ($p = 0.0001$) and overall survival ($p = 0.0001$). In the group of 76 patients classified as TNM stage I, high Ki-67 was the only marker associated with recurrence of disease ($p = 0.05$). The authors concluded that these findings do not support a relevant prognostic role of immunocytochemical markers in NSCLC, even if the Ki-67 index might have particular relevance to identify patients with more aggressive tumors who are at high risk for disease relapse.

The American Thoracic Society’s “Statement on Sarcoidosis” (1999) stated that “In some instances, bronchoalveolar lavage (BAL) and studies on lymphocyte subpopulations are helpful. According to Costabel, a CD4/CD8 ratio greater than 3.5 has a sensitivity of 53 %, a specificity of 94 %, a positive-predictive value of 76 % and a negative-predictive value of 85 %. In other words, a CD4/CD8 ratio greater than 3.5 provides a diagnosis of sarcoidosis with a specificity of 94 % even if the TLB has not been diagnostic”.

Furthermore, an UpToDate review on “Clinical manifestations and diagnosis of pulmonary sarcoidosis” (King, 2015) states that “Bronchoalveolar lavage -- BAL can be used as an adjunctive measure to support the diagnosis of sarcoidosis by demonstrating a reduced number of CD8 cells, an elevated CD4 to CD8 ratio, and an increased amount of activated T cells, CD4 cells, immunoglobulins, and IgG-secreting cells. BAL is also used to exclude infections and malignancy as alternative diagnoses”.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

- **88182**  
  Flow cytometry, cell cycle or DNA analysis

- **88184**  
  Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker

  + **88185**  
    each additional marker (List separately in addition to code for first marker)

- **88187**  
  Flow cytometry, interpretation; 2 to 8 markers

- **88188**  
  9 to 15 markers

- **88189**  
  16 or more markers

ICD-9 codes covered if selection criteria are met:

- **042**  
  Human immunodeficiency virus [HIV] disease [T cell monitoring]

- **151.0 - 151.9**  
  Malignant neoplasm of stomach [gastric, localized without metastatic disease]
164.2 Malignant neoplasm of anterior mediastinum [neuroblastoma, localized without metastatic disease]

164.3 Malignant neoplasm of posterior mediastinum [neuroblastoma, localized without metastatic disease]

182.0 Malignant neoplasm of endometrium [localized without metastatic disease]

183.0 Malignant neoplasm of ovary [localized without metastatic disease]

183.8 Malignant neoplasm of other specified sites of uterine adnexa [localized without metastatic disease]

185 Malignant neoplasm of prostate [localized without metastatic disease]

188.0 - 188.9 Malignant neoplasm of bladder [localized without metastatic disease]

189.0 Malignant neoplasm of renal cell [localized without metastatic disease]

191.0 - 191.9 Malignant neoplasm of brain [medulloblastoma in adults only]

200.00 - 208.91 Malignant neoplasm of lymphatic and hematopoietic tissue

230.2 Carcinoma in situ of stomach [gastric]

233.2 Carcinoma in situ of other and unspecified parts of uterus [endometrial]

233.4 Carcinoma in situ of prostate

233.7 Carcinoma in situ of bladder

233.9 Carcinoma in situ of other and unspecified urinary organs [prostate]

237.5 Neoplasm of uncertain behavior of brain and spinal cord [intracranial in adults only]

238.4 - 238.79 Neoplasm of uncertain behavior, polycythemia vera, histiocytic and mast cells, plasma cells, and other lymphatic and hematopoietic tissues

239.6 Neoplasms of unspecified nature of brain [adults only]

279.00 - 279.9 Disorders involving the immune mechanism [B-cell monitoring for immunosuppressive disorders and primary immunodeficiencies]

282.0 Hereditary spherocytosis
282.5  Sickle-cell trait
282.60 -  Sickle-cell disease
282.69
282.7  Other hemoglobinopathies [hereditary persistence of fetal hemoglobin]
283.2  Hemoglobinuria due to hemolysis from external causes [paroxysmal nocturnal]
289.83  Myelofibrosis
630  Hydatidiform mole
757.33  Congenital pigmentary anomalies of skin [mast cell disease]
795.4  Other nonspecific abnormal histological findings
996.80 - 996.89  Complications of transplanted organ [postoperative monitoring]
V08  Asymptomatic human immunodeficiency virus [HIV] infection status [T cell monitoring]
V42.0 - V42.9  Organ or tissue replaced by transplant [postoperative monitoring]

**ICD-9 code not covered for indications listed in the CPB:**

153.0 - 154.8  Malignant neoplasm of colon and rectum
162.2 - 162.9  Malignant neoplasm of bronchus and lung [non-small cell lung cancer]
174.0 - 175.9  Malignant neoplasm of breast
180.0 - 180.9  Malignant neoplasm of cervix uteri
191.0 - 191.9  Malignant neoplasm of brain [pediatric only]
237.5  Neoplasm of uncertain behavior of brain and spinal cord [pediatric intracranial only]
239.6  Neoplasms of unspecified nature of brain [pediatric only]

**The above policy is based on the following references:**

38. Russo A, Bazan V, Migliavacca M, et al. DNA aneuploidy and high proliferative activity but not K-ras-2 mutations as independent predictors of


