# DNA Ploidy Cell Cycle Analysis

| Policy Number: AHS – M2136 – DNA Ploidy Cell Cycle Analysis | Prior Policy Name and Number, as applicable: |
|---|---|
| Initial Presentation Date: 05/23/2016<br>Revision Date: 06/02/2021 | |

## I. Policy Description

S-phase fraction (SPF) is an assessment of how many cells are actively synthesizing DNA (UIHC, 2016). It is used as a measure of cell proliferation, particularly for cancer (Pinto, André, & Soares, 1999).

## II. Related Policies

| Policy Number | Policy Title |
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## III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request

1. Measurement of flow cytometry-derived DNA content (DNA Index) or cell proliferative activity (S-phase fraction or % S-phase) for prognostic or therapeutic purposes in the routine clinical management of cancers **DOES NOT MEET COVERAGE CRITERIA.**

## IV. Scientific Background

Cancer is the uncontrolled growth and spread of abnormal cells and is increasingly shown to be initiated, propagated, and maintained by somatic genetic events (Johnson et al., 2014). In 2020, an expected 1,806,590 Americans will be diagnosed with new cancer cases, and 606,520 Americans will die from the disease (Siegel, Miller, & Jemal, 2020).

During the cell cycle, DNA synthesis is tightly regulated and only performed just as the cell is about to divide. This step of DNA replication is called the "S-phase" (Christensen, 2021). Dysfunction of DNA replication is significantly associated with cancer, and cancers frequently involve damage or removal of molecular regulators of replication (Van der Aa et al., 2013). Assessment of the fraction of cells in S-phase has been proposed as an indicator of neoplasm aggression. S-phase fraction (SPF) is thought to reflect proliferative activity of cancer and may provide prognostic or therapeutic information (Ermiah et al., 2012). Elevated proliferative activity may predict a worsened disease-free or overall survival in several cancers, such as breast, non-small cell lung, colorectal, ovarian, kidney, bladder, prostate, and endometrial cancers (Bagwell et al., 2001; Gawrychowski, Lackowska, & Gabriel, 2003; Kenney, Zieske, Rinder, & Smith, 2008; Mangili et al., 2008; Pinto et al., 2011; Ross, 1996). However, data supporting the use of SPF as a prognostic tool appears to be inconsistent at best (Locker et al., 2006). Several proprietary tests exist for the assessment of S-phase fraction. For example, NeoGenomics and GenPath both offer tests to evaluate DNA ploidy along with SPF.

*Clinical Validity and Utility*

Dabic et al. (2008) examined flow cytometric parameters (DNA ploidy and SPF) as predictors of survival in cervical adenocarcinoma. The authors defined proliferative activity as the sum of cells in S or G2/M phase and considered proliferative activity above 15% to be "unfavorable." The authors evaluated 51 patients from 1978 to 2004, but the *p*-value for proliferative activity was found to be 0.817, which is not statistically significant. Therefore, the authors concluded that they did not find any association of flow cytometric parameters with patient survival.

Wolfson et al. (2008) studied possible associations between measurements of DNA index (DI), S-phase fraction (SPF), and tumor heterogeneity (TH) using flow cytometry and overall survival for patients with invasive cervical carcinoma treated with definitive irradiation. The investigators examined a total of 57 patients and found 29 to have SPF under 15% and 26 above 15% (with 2 with unknown SPF). However, after a median follow-up of 3.7 years, the authors found no observable 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.

Carloni et al. (2017) evaluated the associations between SPF and peritoneal carcinomatosis from ovarian cancer. Fifty-three patients were examined, and although SPF differed among the different ploidy categories, no significant correlation was found between SPF and clinical pathological characteristics of patients. However, the authors did find that sensitivity to taxol was correlated with SPF, therefore concluding that "ploidy and SPF could facilitate the choice of therapy for patients with peritoneal carcinomatosis (Carloni et al., 2017)."

Svanvik, Stromberg, Holmberg, Marcickiewicz, and Sundfeldt (2019) examined 1113 patients diagnosed with stage I-III grade 1-3 endometrioid endometrial carcinoma in 2006-2011. They evaluated both DNA ploidy and SPF and set the SPF cutoff at 8%. The authors found that 5-year relative survival was significantly associated with SPF and DNA ploidy through a univariate statistical analysis. However, when other variables such as age, grade, and stage were added, SPF and DNA ploidy became statistically insignificant. Therefore, the authors concluded that "S-phase fraction, DNA ploidy, and p53 overexpression did not improve identification of high-risk patients by stage, grade, and age in stage I-III endometrioid endometrial carcinoma (Svanvik et al., 2019)."

Thomas et al. (2020) completed a study to analyze the prognostic implications of DNA repair, DNA ploidy and telomerase in the malignant transformation risk assessment of leukoplakia. Samples from 200 patients with oral leukoplakia, 100 patients with oral cancer and 100 healthy controls were analyzed. The DNA ploidy content was measured with high resolution flow cytometry; the authors identified that "There was significant difference in the distribution of ploidy status, telomerase activity and DNA repair capacity among control, leukoplakia and oral cancer group (p<0.001). When the molecular markers were compared with histological grading of leukoplakia, both DNA ploidy analysis and telomerase activity showed statistical significance (p<0.001) (Thomas et al., 2020)."

Taniguchi et al. investigated the correlation between flow cytometry parameters such as DNA ploidy, DNA index and S-phase fraction and clinical prognostic factors such as mitotic count and Ki-67 labelling index (LI). The cancer of interest was "gastrointestinal stromal tumours (GIST)" and eighteen specimens from laparoscopic local gastrectomy were analyzed. The authors found these flow cytometry parameters to correlate well with mitotic count ≤5 and Ki-67 LI ≤6. DNA index was found to be 83.3% accurate in predicting mitotic count ≤5 and 77.8% accurate in predicting Ki-67 LI ≤6, while S-phase fraction was found to be 94.4% accurate and 88.9% accurate, respectively. The authors concluded that "Rapid flow cytometry parameters can classify risk without the need for histological analysis." (Taniguchi et al., 2021)

## V. Guidelines and Recommendations

**American Society of Clinical Oncology (ASCO) (Harris et al., 2007; Locker et al., 2006)**

The ASCO's updated recommendations on the use of tumor markers in colorectal cancer state that "neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should not be used to determine prognosis of early-stage colorectal cancer" (Locker et al., 2006). The recommendations also state that "as such, flow cytometric determination of DNA ploidy or proliferation should, at best, be considered an experimental tool" (Locker et al., 2006).

In 2007, the ASCO updated the guidelines for the use of tumor markers in breast cancer which noted that there is "insufficient evidence to support routine use in clinical practice of DNA/ploidy by flow cytometry" (Harris et al., 2007).

**National Comprehensive Cancer Network (NCCN) (NCCN, 2021)**

NCCN clinical practice guidelines on diagnosis and/or management of Breast Cancer (Version 2.2021), Cervical Cancer (Version 1.2021), Colon Cancer (Version 2.2021), Small Cell Lung Cancer (Version 2.2021), and Non-Small Cell Lung Cancer (Version 4.2021) do not mention cell proliferation activity (S-phase fraction or % S-phase) as a management tool (NCCN, 2021).

**International Society of Gynecological Pathologists (ISGyP) Endometrial Cancer Project: Guidelines From the Special Techniques and Ancillary Studies Group (Cho et al., 2019)**

These guidelines focus on biomarkers and their potential use for endometrial carcinoma.

The guideline remarks that "Other than markers which are useful in diagnosis, there are few specific studies that provide definitive evidence for the routine use of IHC [immunohistochemistry] or ploidy analysis in determining the prognosis of EC" and that "There is some literature on the association of ploidy with prognosis, with promising results, but there is a lack of definitive studies to determine its true prognostic impact".

Overall, the guideline states that "Clearly, large prospective, well defined, uniform studies are needed to determine the possible role of IHC for specific biomarkers and ploidy analysis in the clinical setting." (Cho et al., 2019)

## VI. State and Federal Regulations, as applicable

Numerous FDA-approved tests exist for the assessment of SPF. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

## VII. Applicable CPT/HCPCS Procedure Codes

| Code Number | Code Description |
|---|---|
| 88182 | Flow cytometry, cell cycle or DNA analysis |



*Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.*

## VIII. Evidence-based Scientific References

Bagwell, C. B., Clark, G. M., Spyratos, F., Chassevent, A., Bendahl, P. O., Stal, O., . . . Baldetorp, B. (2001). Optimizing flow cytometric DNA ploidy and S-phase fraction as independent prognostic markers for node-negative breast cancer specimens. *Cytometry, 46*(3), 121-135. Retrieved from http://dx.doi.org/

Carloni, S., Gallerani, G., Tesei, A., Scarpi, E., Verdecchia, G. M., Virzi, S., . . . Arienti, C. (2017). DNA ploidy and S-phase fraction analysis in peritoneal carcinomatosis from ovarian cancer: correlation with clinical pathological factors and response to chemotherapy. *Onco Targets Ther, 10*, 4657-4664. doi:10.2147/ott.s141117

Cho, K. R., Cooper, K., Croce, S., Djordevic, B., Herrington, S., Howitt, B., . . . Matias-Guiu, X. (2019). International Society of Gynecological Pathologists (ISGyP) Endometrial Cancer Project: Guidelines From the Special Techniques and Ancillary Studies Group. *Int J Gynecol Pathol, 38 Suppl 1*(Iss 1 Suppl 1), S114-s122. doi:10.1097/pgp.0000000000000496

Christensen, K., Hulick, Peter. (2021). Basic genetics concepts: Chromosomes and cell division. Retrieved from https://www.uptodate.com/contents/basic-genetics-concepts-chromosomes-and-cell-division?search=cell%20cycle%20s%20phase&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1#H3312662727

Dabic, M. M., Nola, M., Tomicic, I., Dotlic, S., Petrovecki, M., & Jukic, S. (2008). Adenocarcinoma of the uterine cervix: prognostic significance of clinicopathologic parameters, flow cytometry analysis and HPV infection. *Acta Obstet Gynecol Scand, 87*(3), 366-372. doi:10.1080/00016340801936560

Ermiah, E., Buhmeida, A., Abdalla, F., Khaled, B. R., Salem, N., Pyrhönen, S., & Collan, Y. (2012). Prognostic value of proliferation markers: immunohistochemical ki-67 expression and cytometric s-phase fraction of women with breast cancer in libya. *J Cancer, 3*, 421-431. doi:10.7150/jca.4944

Gawrychowski, J., Lackowska, B., & Gabriel, A. (2003). Prognosis of the surgical treatment of patients with non-small cell lung cancer (NSCLC)--relation to DNA ploidy. *Eur J Cardiothorac Surg, 23*(6), 870-877; discussion 877. Retrieved from http://dx.doi.org/

Harris, L., Fritsche, H., Mennel, R., Norton, L., Ravdin, P., Taube, S., . . . Bast, R. C., Jr. (2007). American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol, 25*(33), 5287-5312. doi:10.1200/jco.2007.14.2364

Johnson, D. B., Dahlman, K. H., Knol, J., Gilbert, J., Puzanov, I., Means-Powell, J., . . . Pao, W. (2014). Enabling a Genetically Informed Approach to Cancer Medicine: A Retrospective Evaluation of the Impact of Comprehensive Tumor Profiling Using a Targeted Next-Generation Sequencing Panel. *Oncologist, 19*(6), 616-622. doi:10.1634/theoncologist.2014-0011

Kenney, B., Zieske, A., Rinder, H., & Smith, B. (2008). DNA ploidy analysis as an adjunct for the detection of relapse in B-lineage acute lymphoblastic leukemia. *Leuk Lymphoma, 49*(1), 42-48. doi:10.1080/10428190701760052

Locker, G. Y., Hamilton, S., Harris, J., Jessup, J. M., Kemeny, N., Macdonald, J. S., . . . Bast, R. C., Jr. (2006). ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol, 24*(33), 5313-5327. doi:10.1200/jco.2006.08.2644

Mangili, G., Montoli, S., De Marzi, P., Sassi, I., Aletti, G., & Taccagni, G. (2008). The role of DNA ploidy in postoperative management of stage I endometrial cancer. *Ann Oncol, 19*(7), 1278-1283. doi:10.1093/annonc/mdn041

NCCN. (2021). NCCN Clinical Practice Guidelines in Oncology. Retrieved from https://www.nccn.org/professionals/physician_gls/default.aspx. Retrieved 3/23/21 https://www.nccn.org/professionals/physician_gls/default.aspx

Pinto, A. E., André, S., & Soares, J. (1999). Short-term significance of DNA ploidy and cell proliferation in breast carcinoma: a multivariate analysis of prognostic markers in a series of 308 patients. *Journal of Clinical Pathology, 52*(8), 604. doi:10.1136/jcp.52.8.604

Pinto, A. E., Pires, A., Silva, G., Bicho, C., Andre, S., & Soares, J. (2011). Ploidy and S-phase fraction as predictive markers of response to radiotherapy in cervical cancer. *Pathol Res Pract, 207*(10), 623-627. doi:10.1016/j.prp.2011.07.007

Ross, J. S. (1996). DNA ploidy and cell cycle analysis in cancer diagnosis and prognosis. *Oncology (Williston Park), 10*(6), 867-882, 887; discussion 887-890. Retrieved from http://dx.doi.org/

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J Clin, 70*(1), 7-30. doi:10.3322/caac.21590

Svanvik, T., Stromberg, U., Holmberg, E., Marcickiewicz, J., & Sundfeldt, K. (2019). DNA ploidy status, S-phase fraction, and p53 are not independent prognostic factors for survival in endometrioid endometrial carcinoma FIGO stage I-III. *Int J Gynecol Cancer*. doi:10.1136/ijgc-2018-000082

Taniguchi, K., Suzuki, A., Serizawa, A., Kotake, S., Ito, S., Suzuki, K., . . . Yamamoto, M. (2021). Rapid Flow Cytometry of Gastrointestinal Stromal Tumours Closely Matches the Modified Fletcher Classification. *Anticancer Res, 41*(1), 131-136. doi:10.21873/anticanres.14758

Thomas, G., Tr, S., George, S. P., Somanathan, T., Sarojam, S., Krishnankutti, N., . . . Ankathil, R. (2020). Prognostic Implications of DNA Repair, Ploidy and Telomerase in the Malignant Transformation Risk Assessment of Leukoplakia. *Asian Pac J Cancer Prev, 21*(2), 309-316. doi:10.31557/apjcp.2020.21.2.309

UIHC. (2016). Cancer diagnostic tests and blood tests word list. Retrieved from https://uihc.org/health-topics/cancer-diagnostic-tests-and-blood-tests-word-list

Van der Aa, N., Cheng, J., Mateiu, L., Zamani Esteki, M., Kumar, P., Dimitriadou, E., . . . Voet, T. (2013). Genome-wide copy number profiling of single cells in S-phase reveals DNA-replication domains. *Nucleic Acids Res, 41*(6), e66. doi:10.1093/nar/gks1352

Wolfson, A. H., Winter, K., Crook, W., Krishan, A., Grigsby, P. W., Markoe, A. M., . . . Lucci, J. A., 3rd. (2008). Are increased tumor aneuploidy and heightened cell proliferation along with heterogeneity associated with patient outcome for carcinomas of the uterine cervix? A combined analysis of subjects treated in RTOG 9001 and a single-institution trial. *Int J Radiat Oncol Biol Phys, 70*(1), 111-117. doi:10.1016/j.ijrobp.2007.05.069