DNA Ploidy Cell Cycle Analysis

Policy Number: AHS – M2136 – DNA Ploidy Cell Cycle Analysis	Prior Policy Name and Number, as applicable:
Initial Presentation Date: 06/01/2021 Revision Date: N/A	

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

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. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx?from2=search1.asp& or the manual website

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 (R. L. 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" (Raby, 2018). 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)."

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, 2020)

NCCN clinical practice guidelines on diagnosis and/or management of Breast Cancer (Version 2.2020), Cervical Cancer (Version 1.2020), Colon Cancer (Version 2.2020), Small Cell Lung Cancer (Version 1.2020), and Non-Small Cell Lung Cancer (Version 3.2020) do not mention cell proliferation activity (Sphase fraction or % S-phase) as a management tool (NCCN, 2020).

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

Billing applicable codes is not a guarantee of payment; see Section III for indications and limitations of coverage that may affect payment

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

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
06-01-2021	Initial presentation