Intraoperative Flow Cytometry
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Definition

Flow Cytometry is an analytical technique with the ability to quantify cell phenotype and to categorize cell populations on the basis of their characteristics. Intraoperative Flow Cytometry (iFC) is the use of flow cytometry during tumor excision for rapid diagnosis of cancer cells and determination of tumor margins.

1. Introduction

Flow Cytometry (FC) is a powerful analytical technique with several applications in phenotypic analysis and the quantification of cellular processes, such as proliferation and cell death [1]. The quantification of state/phenotype of a cell population is among the main advantages of FC over other methods, such as microscopy. Among the main limitations of the methodology is the spectral overlap of fluoroehromes and the inability to detect the intracellular localization of labeled targets. New advances in the field, such as the development of mass cytometry [2][3] and spectral Flow Cytometry [4] allow the simultaneous analysis of several parameters in a single cell, overcoming spectral overlap of traditional cytometry. In addition, Imaging Flow Cytometry [5][6] and more recently imaging mass cytometry [7] combine the analytical potential of cytometry with the imaging abilities of a microscope.

2. Intraoperative Flow Cytometry

In spite of the potential of clinical utility, Flow Cytometric analysis of DNA content and proliferation markers in Central nervous System malignancies (CNSMs) diagnosis have been scarcely used in clinical practice either as a diagnostic or a prognostic tool. This was until the development of a novel concept, which is the intraoperative use of Flow Cytometry for DNA content/ploidy and cell cycle distribution analysis. The development of Intraoperative Flow Cytometry (iFC), during the last decade, offered a novel viewpoint and perspective on the utility of DNA content analysis for the characterization of solid tumors which have not been extensively evaluated, such as hematologic malignancies. The rationale of iFC offered the ability for intraoperative diagnosis, as an alternative to the pathology evaluation of tissue sections obtained during surgery. A modified rapid protocol for cell cycle analysis developed in the University Hospital of Ioannina (Ioannina Protocol) allowed the intraoperative characterization of intracranial lesions and their surgical margins in 6 min per sample. In a study with thirty-one patients, a significant difference in the G0/G1 phase, as well as in S-phase and G2/M fractions between high-grade and low-grade tumors, was demonstrated. In glioblastoma patients, significant differences were found between tumor mass and margins regarding the G0/G1 phase, the S-phase, and (G2/M) tumor fraction (Tumor Index), offering the potential of delineating tumor margins in gliomas [8]. The Ioannina protocol was first established in a retrospective study involving a series of tumor samples taken from 56 patients, during surgery. The results of DNA content analysis showed that the cell cycle distribution analysis could differentiate between grade I from grade II/III meningiomas and low from high grade gliomas. Furthermore, a prognostic significance was found in glioma patients, based on the analysis of clinical results over a 5-year period [9]. Intraoperative cell-cycle analysis of CNSMs, based on the Ioannina protocol has been suggested as an alternative to other novel intraoperative diagnostic techniques, such as mass-spectrometric analysis of tumor metabolites [10][11], the use of 5-Aminolevulinic Acid Fluorescence (5′ALA), and Intraoperative Magnetic Resonance Imaging [12][13][14], as well as intraoperative squish smear cytology [15][16]. Among the advantages are time, sensitivity, and specificity.

A research team from Tokyo Women’s Medical University has independently developed a similar, rapid iFC protocol with an analysis time of 10 min per sample. The results from Shioyama et al., using their iFC
protocol in 328 biopsy specimens of gliomas, revealed an optimal mitotic index of 6.8%, resulting in 88% sensitivity, 88% specificity, 97% positive predictive value, 60% negative predictive value, and 88% diagnostic accuracy. 

A joint publication by members of both groups highlighted that iFC is a promising adjunct for intracranial tumor surgery, may aid the identification of gliomas boundaries, it can identify a tumor’s grade, diagnose lymphoma, and has prognostic value in glioma. As regards prognosis, recent data suggest that the calculation of the malignancy index, based on iFC, may also act as a novel prognostic factor following radiotherapy and chemotherapy with temozolomide.

References


Keywords

central nervous system malignancies;flow cytometry;glioblastoma;intraoperative flow cytometry