Introduction

The accumulation of fluid in serous cavities can represent a systemic complication or local disease [1]. When systemic diseases progress with effusion, the diagnostic and therapeutic approach is usually restricted to the identification and treatment of the underlying cause, such as a pleural effusion associated with congestive heart failure [1] or ascites associated with cirrhosis and portal hypertension [2]. In contrast, local involvement of the pleura and/or peritoneum requires a precise and usually multilaboratory typically intersecional diagnosis.

Malignancy comprises one of the leading causes of exudative cavity effusion [3]. It is estimated that approximately 100,000 to 150,000 individuals per year present malignant pleural effusion in the United States and Europe, of which 50–65% are secondary to lung and breast cancers [4]. Although guided biopsy is the gold standard for demonstrating neoplastic serous involvement, this procedure is not always feasible due to the clinical condition of most patients with advanced disease and the expense involved [5].

Cytological analysis of pleural and/or peritoneal fluid obtained by aspiration is the first step in the diagnosis of malignant effusions. The sensitivity of this method varies from 60% to 96% depending on the type and location of the tumor, the techniques used for preparation and staining, and the cytologist’s expertise in identifying malignant cells [2, 6, 7].

Thus, cytological examination does not provide a definitive diagnosis in up to 40% of cases. Ancillary techniques
using samples obtained by aspiration puncture—a procedure considered minimally invasive and of low-risk—are therefore recommended to improve diagnosis. Diagnostic tools explored in recent decades include the quantification of liquid-soluble tumor markers [7], immunocytochemistry in embedded materials [8], DNA ploidy analysis by flow cytometry [9], and molecular assays such as polymerase chain reaction (PCR) [10].

Aneuploidy is a common finding in neoplastic cells [11], and the demonstration of abnormal cell DNA content is considered indicative of malignancy. Fluorescence in situ hybridization (FISH) has been used in cavity fluids to detect aneuploidy in interphase cells, circumventing the need for cell culture, which could delay the turn-around time (TAT) to result [12, 13].

This study proposes to evaluate the detection of aneuploid cells in pleural and peritoneal fluid samples using the UroVysion® test, originally developed for the diagnosis of bladder cancer [14]. To analyze the diagnostic performance of UroVysion®, we used two different cutoff strategies: (1) the manufacturer cutoff (M-FISH) and (2) a proposed cutoff (P-FISH) developed in this study.

Materials and Methods

Seventy patients with cavitary effusion (pleural and peritoneal) who had been admitted to the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo (HC-FMUSP) were included in the study after providing informed consent. Each sample was representative of one patient. The fluid samples were submitted to conventional biochemistry, microbiology, and cytology examinations for diagnostic evaluation. Routine tests were performed by the HC-FMUSP clinical laboratory, which is accredited by the College of American Pathologists (CAP). The study was approved by the institutional ethics committee.

The variables analyzed were age, gender, and clinical diagnosis according to the International Statistical Classification of Diseases and Related Health Problems (CID), 10th Revision of Codes. Clinical and laboratory data were extracted from medical records and the laboratory system database, respectively. Histopathological diagnosis was considered the gold standard for malignancy. In benign cavity effusions, clinical history, laboratory and imaging examinations, and patient follow-up were used to exclude malignancy.

Cytological examination

After macroscopic sample analysis, nucleated cells were counted in a counting chamber, and the fluid samples were centrifuged (2000 rpm, 10 min) to prepare the slides. Cytological examination (cell differentiation and oncotic cytology) was performed on slides stained with hematological dye (Leishman stain) (Fig. 1).

Based on oncotic cytology, the cases were classified into three categories: “positive,” “suspicious,” or “negative.” In this study, the following conditions were considered “concordant”: (1) “suspicious” or “positive” oncotic cytology and “positive” gold standard; or (2) “negative” oncotic cytology and “negative” gold standard. Light’s criteria [15] for pleural fluid and Rovelstad et al. [16] for peritoneal fluid were used to classify the samples in exudates or transudates.

Molecular cytogenetic study

For cytogenetic analysis, the samples were treated using the commercial multitarget UroVysion FISH kit (Abbott, IL, cat. nº 32-161070) with centromeric alpha probes for chromosomes 3 (CEP® 3 Spectrum red), 7 (CEP7 Spectrum Green), 17 (CEP17 Spectrum Aqua), and the locus-specific probe 9p21 (LSI® p16 Spectrum Gold). The sample was centrifuged (1400 rpm, 5 min), and the pellet obtained was fixed with fresh Carnoy (methanol/acetic acid solution, 3:1 ratio) for slide preparation. The slides were subsequently hybridized with centromeric probes for chromosomes 3 (red-labeled), 7 (green-labeled), 17 (blue-labeled), and the 9p21 region (yellow-labeled). The manufacturer’s instructions were followed with slight

Figure 1. Cytological characteristics of a malignant and a reactive pleural fluid sample. (A) Tumor cells clustering in case of malignant pleural effusion (Leishman); (B) reactive mesothelial clustering of cells in benign pleural effusion (Leishman).
modifications to timing and temperature. The digital images obtained were captured on an Olympus BX41 microscope equipped with a 100 W lamp and fluorescein filters for propidium iodide (FITC-PI, BP 450-490, FL 510, and BP 520, Cat # 487709). The Applied Imaging CytoVision System (San Jose, CA) was used to analyze the images.

A total of 200 interphase cells per sample were analyzed, and only cells with clearly distinguishable signals for monosomy and/or polysomy were counted. A cell was considered aneuploid when marked by the loss or gain of at least two probes (3, 7, or 17) with or not the loss of the 9p21. In the absence of two signals for chromosomes 3, 7, and 17, the cells were considered noninterpretable. Because benign reactive mesothelium can present tetraploidy, cells with these characteristics were excluded from the analysis. This criterion was previously used by Rosolen et al. [13] and Flores-Staino et al. [17] in similar work. The slides were evaluated by two independent observers, and the results represent the average of their measurements.

To classify a case as aneuploid, it is suggested that each laboratory establish its own cutoff for the genetic changes observed. Thus, an effusion was considered aneuploid when the number of abnormal cells was higher than the previously established cutoff (Fig. 2). To establish this value, we analyzed the diploid (normal) and non-diploid (abnormal) signals emitted by cells present in fluid obtained from patients with a known benign effusion. So, for the analytical validation of probe parameters and results interpretation, we used the statistical test of the inverse $\beta$ function (probability in decimal), where $\alpha = 1 + X$ (X represents the highest number of positive signals obtained by the observers) and $\beta = \text{number of cells analyzed}$ [18]. $P$-FISH for the four probes were as follows:

1. Chromosome 3: $>3.0\%$ for one signal or $>3.0\%$ for three or more signals;
2. Chromosome 7: $>4.0\%$ for one signal or $>2.0\%$ for three or more signals;
3. Chromosome 17: $>4.0\%$ for one signal or $>3.0\%$ for three or more signals;
4. Chromosome 9p21: $>4.6\%$ for one signal.

According to the manufacture’s criteria (counting 25 cells), cells were classified as aneuploid when presenting $\geq 4$ gains in the same cell for two or more chromosomes (3, 7, or 17) or $\geq 12$ cells with zero signal for 9p21. The performance of the UroVysion® FISH test was calculated according to the two cutoffs ($M$-FISH and $P$-FISH).

### Statistical analyses

Continuous variables are described by the median, mean, and standard deviation (SD). Categorical variables are presented as a percentage. A comparison of the performance of UroVysion® FISH with both cutoffs was made by chi-square test or Student’s $t$ test. The significance
level $P < 0.05$ was adopted. Contingency analyses were performed to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Data were analyzed using the Microsoft Office 365 Excel programs (Redmond, WA) and the 17th version of Minitab Statistical Software (Minitab Inc. International Sales and Support, State College, PA). Figure 3 summarizes the study design.

**Table 1.** Characteristics of the study group and of the fluid samples.

| Patients ($N$) | 70 |
|---------------|----|
| Age           | 62.8 ± 15.0 |
| Male/Female ($N$) | 26/44 |
| Pleural/Peritoneal ($N$) | 60/10 |
| Aspect Before centrifugation | Yellow/Ser-H/Hemorrhagic/Brownish/Colorless/Purulent |
| Color Before centrifugation | Clear/slightly cloudy/cloudy |
| Cell Count (mm$^3$) | Median 755 |
| Neutrophils/Lymphocytes predominance ($N$) | 21/49 |
| Cytology Positive/suspicious/negative | 40/15/15 |
| Transudate/Exudate ($N$)$^1$ | 15/45 |
| ADA ± SD | 11.4 ± 9.3 |
| Positive culture ($N$) | 3/0/0 |

ADA, adenosine deaminase; $N$, number; SD, standard deviation; Ser-H, serum-hemorrhagic.

$^1$There were 10 cases with insufficient data for classification.

**Figure 4.** Tumor primary sites in cases with malignant effusion.

**Results**

Seventy patients with pleural or peritoneal effusion were included: 63 (90%) with malignant etiology and seven (10%) of benign origin. From the malignant effusions, positive cytology was observed in 40 (63.5%), negative in nine (14.3%), and suspicious in 14 (22.2%) cases. The general characteristics of the study group and of the fluids are shown in Table 1.

In malignant effusions, the most common tumor primary sites were breast and lung, with less representation of the other sites (Fig. 4). Hematological malignancies were represented by seven cases of lymphoma and one case of multiple myeloma. Of the benign effusions, three cases were cardiovascular system diseases, two were tuberculosis, one was cirrhosis, and one was chronic kidney disease.

In most cases of aneuploidy, a predominance of chromosomal gains was observed. In all cases, it was possible to count 200 cells/case. The signal frequencies for each probe in the $P$-FISH analysis are shown in Figure 5.

The performance of cytology—considered the gold standard for etiological diagnosis—and FISH (with proposed and manufacturer cutoffs) is shown in Tables 2–4.
Discussion

In the present study, UroVysion® FISH using the manufacturer’s cutoff performed worse than with the proposed cutoff for the identification of malignant effusions. Using the proposed cutoff, the diagnostic sensitivity was 87.3%, with an accuracy of 85.7%, a PPV of 96.4%, and a specificity of 71.4%. When combined with cytology, the sensitivity was 88.0%, with an accuracy of 87.8%, and an improved specificity of 83.3%. P-FISH was the only examination that identified two cases of malignant pleural effusion secondary to ovarian and lung cancer. However, it failed to identify a case of malignant pleural effusion due to myelomatous infiltration.
CHROMOSOME 3

Loss of p25-pter is associated with distant metastasis and poor survival in colorectal cancer

Mutation of p24-p25 in squamous cell carcinoma of the tongue

Deletions p21-p23 associated small cells lung cancer

Expression loss of several genes from both LUCA and AP20 subregions within the p21.3 locus in non-small-cells lung cancer

Homozogous deletion, rearrangement, and hypermethylation of the p14-p21 region associated with breast cancer

The p11-12 locus associated with invasive bladder cancer

Loss of heterozygosity at the short arm in renal cell cancer

The genes LRRN1, PRICKLE2, VHL, BHLHE40, RBSP3, and CGGBP1 e SOX14 altered in squamous cell carcinomas

Uveal melanoma is associated with mmosomy

Mutation in the Von Hippel–Lindau gene in the p25 region in renal cell cancer

FHIT gene mutation for prostate cancer risk

Mutation of the SPARC, DAB2, and VEGF gene in ovarian cancer

Rearrangement in band q13.2 in small cells lung cancer

CHROMOSOME 7

Deleted region on the long arm in stomach cancer

Mutations associated with prostate cancer

Polysoy associated with a higher incidence of axillary lymph node metastasis, poorly differentiated (grade III) in breast cancer

Polysoy associated with negative estrogen and progesterone receptor and a higher Ki67 labeling index in breast cancer

Rearrangement of regions p13, q34 possibly associated with hematological cancer

Translocations in the q32-35 region in leukemia and T-cell lymphoma

CHROMOSOME 9p21

Deletion associated with worse prognosis in prostate cancer

Loss of heterozygosity is associated with aggressive renal tumors

INK4A/ARF/INK4B locus deletion in melanoma, pancreatic adenocarcinoma, glioblastoma, leukemias, non-small-cell lung cancer, and bladder carcinoma

Loss of p16 in the pathogenesis of malignant peripheral nerve sheath tumors

Loss of p16 (INK4A) in the development of synovial sarcoma

Loss of heterozygosity in enteropathy-type T-cell lymphoma

Deletions associated with malignant progression of meningiomas and poor prognosis in anaplastic meningiomas

Tobacco and the loss of the locus p16 in the pathogenesis of lung cancer

Loss is an early event in head and neck squamous cell carcinoma

CHROMOSOME 17

Percentage of hyperdiploid cells correlates with overexpression of p53, tumor grade increase and advanced pathological stage in bladder cancer

C-erbB-2 (q21) encodes a transmembrane glycoprotein like the epidermal growth factor receptor in bladder cancer

Deletion p53 and amplification of C-erbB-2 associated with worse prognosis in breast cancer

Deletion p12-p13.3 (region contains the gene for the transformation-associated protein p53) associated with colorectal cancer

Gains in LASP2 (q12), TGF11 (q21.32), MUL (q23.2), TBX2 (q23.2), AXIN2 (q24.3), and GAB2 (q25.1) associated with ovarian cancer

Decreased NM23 metastasis suppressor gene expression in breast cancer

Numerical abnormalities associated with rectal cancer

The hereditary predisposition for breast cancer related to the BRCA-1 gene

Figure 6. Chromosomal abnormalities observed on chromosomes 3, 7, 17, and 9p21.
The clinical presumption of malignant effusion in patients with or without prior history of cancer poses a challenge to the cytologist, especially in cases where cytology, although atypical, is inconclusive. Thus, complementary examinations of the pleural or peritoneal fluid should be considered potential tools for diagnosis without adding further risk to the patient. In this context, new methodologies such as proteomic assays have shown promising results (for example, CARD9—isoform 1 of caspase recruitment domain member 9), but have limitations and are not widely used [19]. In the same way, the detection of circulating tumor cells (CTCs) in liquid biopsies has emerged as a tool with great diagnostic and prognostic potential, spawning new clinical trials using blood, urine, saliva, feces, sputum, cavity, and cerebrospinal fluid for the diagnosis and monitoring of patients with cancer, mainly by detecting copy number variation of genes by next-generation sequencing (NGS) or FISH assay [20].

The FISH assay is widely used in the clinical laboratory and can be applied in the evaluation of chromosomal abnormalities in nondividing cells, with results available within 24 h [21]. In the present study, this cytogenetic technique was used to recognize numerical DNA alterations in genomic regions of interest for oncology without significantly altering the turnaround time (TAT) to diagnosis. Aneuploidy is triggered by a high rate of single chromosomal missegregation, as seen in the chromosomal instability and inactivation of the p53 pathway [11]. In cavity fluid, abnormal cellular DNA content may be an important indicator of malignancy, especially in cases in which cytology does not allow for definitive diagnosis [22]. In our study, we evaluated the detection of aneuploid cells in effusions with the UroVysion® FISH test, which was originally developed for the urine diagnosis and follow-up of patients with bladder cancer. The UroVysion® FISH test consists of four labeled probes that hybridize to the centromeric regions of chromosomes 3, 7, and 17, as well as to the 9p21 locus, which are considered potential targets for carcinogenesis and the development of metastasis [23-57]. Figure 6 details the major chromosomal abnormalities present in these target regions.

Although we obtained satisfactory results with the UroVysion® test in the diagnosis of malignant effusions, data in the literature show varying sensitivity, specificity, PPV, and NPV values in the diagnosis and follow-up of patients with urinary tract tumors (mainly bladder cancer) and of patients with tumors in the bile ducts and pancreas, as can be observed in Table 5. Flores-Staino et al. [17] reported chromosomal aberrations in 29 samples of pleural fluid from patients with metastatic carcinoma using the UroVysion® test. Cora et al. [22], Ioakim-Liossi et al. [58], Roka et al. [59], and Fiegl et al. [60] also used the FISH assay to investigate chromosomal aberrations in cavity fluids using other protocols and probes for different chromosomes.

### Table 5. Literature data describing the use of UroVysion® FISH in several cancer types.

| Tumor                                | N | S (%) | E (%) | PPV (%) | NPV (%) |
|--------------------------------------|---|-------|-------|---------|---------|
| Liew et al. [61]                     | 30| 84.2  | 100   | 100     | 65.4    |
| Virk et al. [62]                     | 377| 44.6  | 81.8  | 47.1    | 80.2    |
| Lavery et al. [63]                   | 129| 67    | 76    | —       | —       |
| Gomella et al. [64]                  | 415| 50/51.9| 69.9/89.3| 40.3/90 | 77.4/50 |
| Gopalakrishna et al. [65]            | 2040| 37    | 84    | —       | —       |
| Mischinger et al. [66]               | 1048| 71.9  | 69.3  | 39.4    | 89.9    |
| Miki et al. [67]                     | 91 | 62.5  | 100   | 100     | 85.7    |
| Zhou et al. [68]                     | 1532| 78.9/65.9 | 59.2/78.9 | 77/84.41 | 61.8/57.11 |
| Dudley et al. [69]                   | 72 | 55    | 94    | —       | —       |
| Glass et al. [70]                    | 942| 55.1  | 78.7  | —       | —       |
| Fritcher et al. [71]                 | 272| 46    | 91    | —       | —       |
| Fritsche et al. [72]                 | 210| 95    | 93    | 76      | 99      |
| Breen et al. [73]                    | 939| 47.7  | 87.7  | —       | —       |
| Todenhöfer et al. [74]               | 483| 74.3  | 69.6  | 46.8    | 88.2    |
| Vlajic et al. [75]                   | 90 | 26.7  | 100   | 100     | 63.3    |
| Ho et al. [76]                       | 627| 89.2  | 83.4  | 47.1    | 97.9    |
| Dimashkieh et al. [77]               | 1835| 61.9  | 89.7  | 53.9    | 87.5    |
| Todenhöfer et al. [77]               | 2365| 70.8/61.5| 80.1/80.1| —       | —       |
| Youssef et al. [78]                  | 123| 23.5  | 94.3  | 40      | 88.5    |
| Caraway et al. [79]                  | 1006| 61    | 58    | 42      | 79      |
| Mian et al. [80]                     | 55 | 100   | 89.5  | 84.6    | 100     |
| Kehinde et al. [81]                  | 178| 80    | 48    | 61      | 71.2    |

E, specificity; N, number; NPV, negative predictive value; PPV, positive predictive value; S, sensitivity.

Criteria considered with tetrasomy/without tetrasomy.
Considering that the majority of patients with malignant effusions present advanced disease with significant systemic impairment [4], the possibility of establishing a cancer diagnosis using aspirated fluid samples is of great value in clinical practice, as it avoids submitting patients to invasive diagnostic procedures. In this study, the increase in diagnostic sensitivity with P-FISH was not significant when compared to cytology. However, the assay was effective in demonstrating aneuploidy and, therefore, in confirming malignancy in all cases of suspicious cytology (14 cases). It is important to emphasize that this study is the first to propose a different cutoff value for the diagnosis of malignant pleural or peritoneal effusion with the UroVysion® test, including samples from patients with metastases of solid tumors and hematological malignancies.

However, although the results are promising, we must highlight some limitations of the study: (1) the absence of malignant mesothelioma in the study casuistic, as the homozygous deletion of the 9p21 gene is more frequently observed in this type of tumor; (2) the small number of benign effusions, which are important for validation of assay specificity; and (3) the small number of hematological malignancy cases included.

In conclusion, the present study showed that the UroVysion® P-FISH was effective in the identification of aneuploid cells in cavity fluids of patients with malignant effusions. UroVysion® P-FISH exhibited good sensitivity and accuracy, especially in cases of inconclusive cytology. However, for use in clinical practice, a greater number of effusions should be evaluated, including a wider spectrum of malignancies known to evolve with cavitary effusions.

Conflict of Interest
The authors declare no conflict of interests.

References
1. Froudarakis, M. E. 2008. Diagnostic work-up of pleural effusions. Respiration 75:4–13.
2. Peter, S., I. Eltoum, and M. A. Eloubeidi. 2017. EUS-guided FNA of peritoneal carcinomatosis in patients with unknown primary malignancy. Gastrointest. Endosc. 70:1266–1270.
3. Karpathiou, G., D. Stefanou, and M. E. Froudarakis. 2015. Pleural neoplastic pathology. Respir. Med. 109:931–943.
4. Penz, E., K. N. Watt, and C. A. Hergott. 2017. Management of malignant pleural effusion: challenges and solutions. Cancer Manag. Res. 9:229–241.
5. Rodriguez-Panadero, F., J. P. Janssen, and P. Astoul. 2006. Thoracoscopy: general overview and place in the diagnosis and management of pleural effusion. Eur. Respir. J. 28:409–422.
6. Johnston, W. W. 1985. The malignant pleural effusion. A review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. Cancer 56:905–909.
7. Antonangelo, L., R. K. Sales, A. P. Corá, M. M. P. Acencio, L. R. Teixeira, and F. S. Vargas. 2015. Pleural fluid tumour markers in malignant pleural effusion with inconclusive cytologic results. Curr. Oncol. 22:336–341.
8. Fetsch, P. A., and A. Abati. 2001. Immunocytochemistry in effusion cytology: a contemporary review. Cancer 93:293–308.
9. Laerum, O. P., and T. Farsund. 1981. Clinical applications of flow cytometry: a review. Cytometry 1981:1–13.
10. Sakaguchi, M., A. K. Virmani, and R. Ashfaq. 1999. Development of a sensitive, specific reverse transcriptase polymerase chain reaction-based assay for epithelial tumour cells in effusions. Br. J. Cancer 79:416–422.
11. Thompson, S. L., and D. A. Compton. 2010. Proliferation of aneuploid human cells is limited by a p53-dependent mechanism. J. Cell Biol. 188:369–381.
12. Savic, S., N. Franco, B. Grilli, A. de Vito Barascud, M. Herzog, B. Bode, et al. 2010. Fluorescence in situ hybridization in the definitive diagnosis of malignant mesothelioma in effusion cytology. Chest 138:137–144.
13. Rosolen, D. C., L. D. Kulikowski, G. Bottura, A. M. Nascimento, M. Acencio, L. R. Teixeira, et al. 2013. Efficacy of two fluorescence in situ hybridization (FISH) probes for diagnosing malignant pleural effusions. Lung Cancer 80:284–288.
14. Dimashkieh, H., D. J. Wolff, T. M. Smith, P. M. Houser, P. J. Nietert, and J. Yang. 2013. Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. Cancer Cytopathol. 121:591–597.
15. Light, R. W. 2002. Clinical practice. Pleural effusion. N. Engl. J. Med. 346:1971–1977.
16. Rovelstad, R. A., L. G. Bartholomew, and J. C. Cain. 1958. Ascites. I. The value of examination of ascitic fluid and blood for lipids and for proteins by electrophoresis. Gastroenterology 34:436–451.
17. Flores-Staino, C., E. Darai-Ramqvist, K. Dobra, and A. Hjerpe. 2010. Adaptation of a commercial fluorescent in situ hybridization test to the diagnosis of malignant tumour cells in effusions. Lung Cancer 68:39–43.
18. Wolff, D. J., A. Bagg, L. D. Cooley, G. W. Dewald, B. A. Hirsch, P. B. Jacky, et al. 2007. Guidance for fluorescence in situ hybridization testing in hematologic disorders. J. Mol. Diagn. 9:134–143.
19. Li, H., Z. Tang, H. Zhu, H. Ge, S. Cui, and W. Jiang. 2016. Proteomic study of benign and malignant pleural effusion. J. Cancer Res. Clin. Oncol. 142:1191–1200.
20. Siravegna, G., S. Marsoni, S. Siena, and A. Bardelli. 2017. Integrating liquid biopsies into the management of cancer. Nat. Rev. Clin. Oncol. 14:531–548.
21. Faggioli, F., J. Vijg, and C. Montagna. 2014. Four-color FISH for the detection of low-level aneuploidy in interphase cells. Methods Mol. Biol. 1136:291–300.
22. Cora, T., H. Acar, S. Ceran, and S. Bodur. 2005. Analysis of chromosomes 9 and 11 aneuploidy frequency in pleural effusion of patients with and without malignancy: interphase FISH technique. Cancer Biol. Ther. 4:248–251.
23. Tsai, M. H., W. H. Fang, S. H. Lin, S. T. Tzeng, C. S. Huang, S. J. Yen, et al. 2011. Mapping of genetic deletions on chromosome 3 in colorectal cancer: loss of 3p25-pter is associated with distant metastasis and poor survival. Ann. Surg. Oncol. 18:2662–2670.
24. Arai, K., T. Shibahara, N. Yamamoto, T. Yakushiji, C. Tanaka, and H. Noma. 2001. Frequent allelic loss/imbalance on the short arm of chromosome 3 in tongue cancer. Bull. Tokyo Dent. Coll. 42:151–157.
25. Mooibroek, H., J. Oisinga, P. E. Postmus, B. Carritt, and C. H. Buys. 1987. Loss of heterozygosity for a chromosome 3 sequence presumably at 3p21 in small cell lung cancer. Cancer Genet. Cyto. 27:361–365.
26. Senchenko, V. N., E. A. Anedchenko, T. T. Kondratieva, G. S. Krasnov, A. A. Dmitriev, V. I. Zabarovska, et al. 2010. Simultaneous down-regulation of tumor suppressor genes RB1, RB3P3, CTDSPL, NPR1L2, G21 and RASSF1A in primary non-small cell lung cancer. BMC Cancer 10:75.
27. Buchhagen, D. L., L. Qiu, and P. Etkind. 1994. Homozygous deletion, rearrangement and hypermethylation implicate chromosome region 3p14.3-3p21.3 in sporadic breast-cancer development. Int. J. Cancer 57:473–479.
28. Wada, T., J. Louhelainen, K. Hemminke, J. Adolfsson, H. Wijkström, U. Norming, et al. 2001. The prevalence of loss of heterozygosity in chromosome 3, including FHIT, in bladder cancer, using the fluorescent multiplex polymerase chain reaction. BJU Int. 87:876–881.
29. Woodward, E. R., A. B. Skytte, D. G. Cruger, and E. R. Maher. 2010. Population-based survey of cancer risks in chromosome 3 translocation carriers. Genes Chromosom. Cancer. 49:52–58.
30. Senchenko, V. N., N. P. Kisseljova, T. A. Ivanova, A. A. Dmitriev, G. S. Krasnov, A. V. Kudryavtseva, et al. 2013. Novel tumor suppressor candidates on chromosome 3 revealed by Notil-microarrays in cervical cancer. Epigenetics 8:409–420.
31. Sipos, E., K. Hegyi, A. Treszl, Z. Steiber, G. Mehes, N. Dobos, et al. 2017. Concordence of chromosome 3 and 4 aberrations in human uveal melanoma. Oncol. Rep. 37:1927–1934.
32. Lubinski, J., P. Hadaczek, J. Podolski, A. Toloczko, A. Sikorski, P. McCue, et al. 1994. Common regions of deletion in chromosome regions 3p12 and 3p14.2 in primary clear cell renal carcinomas. Cancer Res. 54:3710–3713.
33. Larson, G. P., Y. Ding, L. S. Cheng, C. Lundberg, V. Gagalang, G. Rivas, et al. 2005. Genetic linkage of prostate cancer risk to the chromosome 3 region bearing FHIT. Cancer Res. 65:805–814.
34. Cody, N. A., V. Ouellet, E. N. Manderson, M. C. J. Quinn, A. Filali-Mouhim, P. Tellis, et al. 2007. Transfer of chromosome 3 fragments suppresses tumorigenicity of an ovarian cancer cell line monoallelic for chromosome 3p. Oncogene 26:618–632.
35. Dennis, T. R., and A. D. Stock. 1999. A molecular cytogenetic study of chromosome 3 rearrangements in small cell lung cancer: consistent involvement of chromosome band 3q13.2. Cancer Genet. Cyto. 113:134–140.
36. Nishizuka, S., G. Tamura, M. Terashima, and R. Satodate. 1997. Commonly deleted region on the long arm of chromosome 7 in differentiated adenocarcinoma of the stomach. Br. J. Cancer 76:1567–1571.
37. Kasahara, K., T. Taguchi, I. Yamasaki, T. Karashima, M. Kamada, K. Yuri, et al. 2001. Fluorescence in situ hybridization to assess transitional changes of aneuploidy for chromosomes 7, 8, 10, 12, 16, X and Y in metastatic prostate cancer following anti-androgen therapy. Int. J. Oncol. 19:543–549.
38. Kapranos, N., S. Kounelis, H. Karantasis, and E. Kouri. 2005. Numerical aberrations of chromosomes 1 and 7 by fluorescent in situ hybridization and DNA ploidy analysis in breast cancer. Breast J. 11:448–453.
39. Scheres, J. M., T. W. J. Hustinx, and J. M. Trent. 1986. Possible involvement of unstable sites on chromosomes 7 and 14 in human cancer. Cancer Genet. Cyto. 19:151–158.
40. Raimondi, S. C., C. H. Pui, and F. G. Behm. 1987. 7q32-q36 Translocations in childhood T cell Leukemia: cytogenetic Evidence for Involvement of the T cell Receptor/9- chain Gene. Blood 1:131–134.
41. Barros, E. A. F., J. Pontes-Junior, and S. T. Reis. 2017. Correlation between chromosome 9p21 locus deletion and prognosis in clinically localized prostate cancer. Int. J. Biol. Markers 32:e248–e254.
42. El-Mokadem, L., A. Lim, T. Kidd, K. Garret, N. Pratt, D. Batt, et al. 2016. Microsatellite alteration and immunohistochemical expression profile of chromosome 9p21 in patients with sporadic renal cell carcinoma following surgical resection. BMC Cancer 16:546.
43. Kim, W. Y., and N. E. Sharpless. 2006. The regulation of INK4/ARF in cancer and aging. Cell 127:265–275.
44. Sabah, M., R. Cummins, M. Leader, and E. Kay. 2006. Loss of p16 (INK4A) expression is associated with allelic imbalance/loss of heterozygosity of chromosome 9p21 in microdissected malignant peripheral nerve.
sheath tumors. Appl. Immunohistochem. Mol. Morphol. 14:97–102.
45. Sabah, M., R. Cummins, M. Leader, and E. Kay. 2005. Loss of p16INK4A expression is associated with allelic imbalance/loss of heterozygosity of chromosome 9p21 in microdissected synovial sarcomas. Virchows Arch. 447:842–848.
46. Obermann, E. C., T. C. Diss, R. A. Hamoudi, P. Munson, B. S. Wilkins, M. L. P. Camozzi, et al. 2004. Loss of heterozygosity at chromosome 9p21 is a frequent finding in enteropathy-type T-cell lymphoma. J. Pathol. 202:252–262.
47. Banerjee, R., C. M. Lohse, B. K. Kleinschmidt-DeMasters, and B. W. Scheithauer. 2002. A role for chromosome 9p21 deletions in the malignant progression of meningiomas and the prognosis of anaplastic meningiomas. Brain Pathol. 12:183–190.
48. Sanchez-Cespedes, M., P. A. Decker, K. M. Doffek, M. Esteller, W. H. Westra, E. A. Alawi, et al. 2001. Increased loss of chromosome 9p21 but not p16 inactivation in primary non-small cell lung cancer from smokers. Cancer Res. 61:2092–2096.
49. Van Der Riet, P., H. Nawroz, R. H. Hruban, R. Corio, K. Tokino, W. Koch, et al. 1994. Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. Cancer Res. 54:1156–1158.
50. Li, B., H. Kanamaru, S. Noriki, M. Fukuda, and K. Okada. 1998. Numeric aberration of chromosome 17 is strongly correlated with p53 overexpression, tumor proliferation and histopathology in human bladder cancer. Int. J. Urol. 5:317–323.
51. Miyamoto, H., Y. Kubota, S. Noguchi, K. Takase, J. Matsuzaki, M. Moriyama, et al. 2000. C-ERBB-2 gene amplification as a prognostic marker in human bladder cancer. Urology 55:679–683.
52. Hislop, R. G., N. Pratt, S. C. Stocks, C. M. Steel, M. Sales, D. Goudie, et al. 2002. Karyotypic aberrations of chromosomes 16 and 17 are related to survival in patients with breast cancer. Br. J. Surg. 89:1581–1586.
53. Kawai, M., H. Komiyama, M. Hosoya, H. Okubo, T. Fujii, N. Yokoyama, et al. 2016. Impact of chromosome 17q deletion in the primary lesion of colorectal cancer on liver metastasis. Oncol. Lett. 12:4773–4778.
54. Dimova, I., B. Orsetti, V. Negre, C. Rouge, L. Ursule, L. Lasorsa, et al. 2009. Genomic markers for ovarian cancer at chromosomes 1, 8 and 17 revealed by array CGH analysis. Tumori 95:357–366.
55. Rinker-Schaeffer, C. W., A. L. Hawkins, N. Ru, J. Dong, G. Stoica, C. A. Griffin, et al. 1994. Differential suppression of mammary and prostate cancer metastasis by human chromosomes 17 and 11. Cancer Res. 54:6249–6256.
56. Garcia, J., A. Duran, M. D. Tabenero, A. Garcia Plaza, T. Flores Corral, M. L. Najera, et al. 2003. Numerical abnormalities of chromosomes 17 and 18 in sporadic colorectal cancer: incidence and correlation with clinical and biological findings and the prognosis of the disease. Cytotherapy B Clin. Cytom. 51:14–20.
57. Forsti, A., L. Luo, I. Vorechovsky, M. Soderberg, P. Lichtenstein, and K. Hemminki. 2001. Allelic imbalance on chromosomes 13 and 17 and mutation analysis of BRCA1 and BRCA2 genes in monozygotic twins discordant for breast cancer. Carcinogenesis 22:27–33.
58. Ioakim-Liossi, A., S. Gagos, P. Athanassiadis, P. Athanassiadou, J. Gogas, P. Davaris, et al. 1999. Changes of chromosomes 1, 3, 6 and 11 in metastatic effusions arising from breast and ovarian cancer. Cancer Genet. Cytogenet. 110:34–40.
59. Roka, S., M. Fiegli, N. Zojer, M. Filipits, R. Schuster, B. Steinier, et al. 1998. Aneuploidy of chromosome 8 as detected by interphase fluorescence in situ hybridization is a recurrent finding in primary and metastatic breast cancer. Breast Cancer Res. Treat. 48:125–133.
60. Fiegli, M., M. Haun, A. Massoner, J. Kruemnann, E. Müller-Holzner, R. Hack, et al. 2004. Combination of cytology, fluorescence in situ hybridization for aneuploidy, and reverse-transcriptase polymerase chain reaction for human mammaglobin/mammaglobin B expression improves diagnosis of malignant effusions. J. Clin. Oncol. 22:474–483.
61. Liew, Z. H., T. J. Z. Loh, T. K. H. Lim, T. H. Lim, C. J. L. Khor, S. J. Mesenas, et al. 2018. Role of fluorescence in situ hybridization in diagnosing cholangiocarcinoma in indeterminate biliary strictures. J. Gastroenterol. Hepatol. 33:315–319.
62. Virk, R. K., S. Abro, J. M. M. de Ubago, S. E. Pambuccian, M. L. Quek, E. M. Wojcik, et al. 2017. The value of the UroVysion® FISH assay in the risk-stratification of patients with “atyptical urothelial cells” in urinary cytology specimens. Diagn. Cytopathol. 45:481–500.
63. Laverty, H. J., B. Zaharieva, and A. McFaddin. 2017. A prospective comparison of UroVysion FISH and urine cytology in bladder cancer detection. BMC Cancer 17:247.
64. Comella, L. G., M. J. Mann, R. C. Cleary, S. G. Hubosky, D. H. Bagley, A. B. Thumar, et al. 2017. Fluorescence in situ hybridization (FISH) in the diagnosis of bladder and upper tract urothelial carcinoma: the largest single-institution experience to date. Can. J. Urol. 24:8620–8626.
65. Gopalakrishna, A., J. J. Fantony, and T. A. Longo. 2017. Anticipatory positive urine tests for bladder cancer. Ann. Surg. Oncol. 24:1747–1753.
66. Mischinger, J., L. P. Guttenberg, J. Hennenlotter, G. Gakis, S. Auferklamm, S. Rausch, et al. 2017. Comparison of different concepts for interpretation of chromosomal aberrations in urothelial cells detected by
fluorescence in situ hybridization. J. Cancer Res. Clin. Oncol. 143:677–685.
67. Miki, Y., M. Neat, and A. Chandra. 2017. Application of The Paris System to atypical urine cytology samples: correlation with histology and UroVysion® FISH. Cytopathology 28:88–95.
68. Zhou, A. G., Y. Liu, and M. S. Cyr. 2016. Role of tetrasomy for the diagnosis of urothelial carcinoma using UroVysion fluorescent in situ hybridization. Arch. Pathol. Lab. Med. 140:552–559.
69. Dudley, J. C., Z. Zheng, T. McDonald, L. P. Le, D. Dias-Santagata, D. Borger, et al. 2016. Next-generation sequencing and fluorescence in situ hybridization have comparable performance characteristics in the analysis of pancreaticobiliary brushings for malignancy. J. Mol. Diagn. 18:124–130.
70. Glass, R. E., C. Coutsouvelis, S. Sheikh-Fayyaz, K. Chau, L. Rosen, R. Brenkert, et al. 2016. Two-tiered subdivision of atypia on urine cytology can improve patient follow-up and optimize the utility of UroVysion. Cancer Cytopathol. 124:188–195.
71. Fritcher, E. G. B., J. S. Voss, S. M. Brankley, M. B. Campion, S. M. Jenkins, M. E. Keeney, et al. 2015. An optimized set of fluorescence in situ hybridization probes for detection of pancreaticobiliary tract cancer in cytology brush samples. Gastroenterology 149:1813–1824.
72. Fritsche, H. M., M. Burger, W. Dietmaier, S. Denzinger, E. Bach, W. Otto, et al. 2010. Multicolor FISH (UroVysion) facilitates follow-up of patients with high-grade urothelial carcinoma of the bladder. Am. J. Clin. Pathol. 134:597–603.
73. Breen, V., N. Kasabov, A. M. Kamat, E. Jacobson, J. M. Suttie, P. J. O’Sullivan, et al. 2015. A holistic comparative analysis of diagnostic tests for urothelial carcinoma: a study of Cxbladder Detect, UroVysion® FISH, NMP22® and cytology based on imputation of multiple datasets. BMC Med. Res. Methodol. 15:45.
74. Todenhöfer, T., J. Hennenlotter, M. Esser, S. Mohrhardt, S. Außerklamm, J. Böttge, et al. 2014. Stepwise application of urine markers to detect tumor recurrence in patients undergoing surveillance for non-muscle-invasive bladder cancer. Dis. Markers 2014:973406.
75. Vlajnic, T., G. Somaini, S. Savic, A. Barascud, B. Grilli, M. Herzog, et al. 2014. Targeted multiprobe fluorescence in situ hybridization analysis for elucidation of inconclusive pancreaticobiliary cytology. Cancer Cytopathol. 122:627–34.
76. Ho, C. C., W. P. Tan, R. Pathmanathan, W. K. Tan, and H. M. Tan. 2013. Fluorescence-in-situ-hybridization in the surveillance of urothelial cancers: can use of cystoscopy or ureteroscopy be deferred? Asian Pac. J. Cancer Prev. 14:4057–4059.
77. Todenhöfer, T., J. Hennenlotter, V. Tews, G. Gakis, S. Außerklamm, U. Kuehs, et al. 2013. Impact of different grades of microscopic hematuria on the performance of urine-based markers for the detection of urothelial carcinoma. Urol. Oncol. 31:1148–1154.
78. Youssef, R. F., B. J. Schlomer, R. Ho, A. I. Sagalowsky, R. Ashfaq, and Y. Lotan. 2012. Role of fluorescence in situ hybridization in bladder cancer surveillance of patients with negative cytology. Urol Oncol. 30:273–7.
79. Caraway, N. P., A. Khanna, R. L. Fernandez, L. Payne, R. L. Bassett, H. Z. Zhang, et al. 2010. Fluorescence in situ hybridization for detecting urothelial carcinoma: a clinicopathologic study. Cancer Cytopathol. 118:259–268.
80. Mian, C., G. Mazzoleni, S. Vikoler, T. Martini, R. Knüchel-Clark, D. Zaak, et al. 2010. Fluorescence in situ hybridization in the diagnosis of upper urinary tract tumours. Eur. Urol. 58:288–292.
81. Kehinde, E. O., F. Al-Mulla, K. Kapila, and J. T. Anim. 2011. Comparison of the sensitivity and specificity of urine cytology, urinary nuclear matrix protein-22 and multitarget fluorescence in situ hybridization assay in the detection of bladder cancer. Scand. J. Urol. Nephrol. 45:113–121.