a power of 0.8 using exact test and with an associated false discovery rate of 0.1.

Results. To identify differential genes expression levels, we collected and analyzed 48 biospecimes (16 normal tissues, 16 preneoplastic tissues, 16 cancer tissues) from 16 patients (8 HPV+ VSCC, 8 HPV- VSCC) with a mean age of 71 (±11) for HPV+ group, and 73 (±10) for HPV-. We normalized raw counts and removed from analysis samples with less than 1,000,000 reads (7 samples). Transcripts with less than 5 reads per million in 30% of samples were filtered from the dataset, and remaining genes were log2-transformed. We evaluated differential gene expression levels in tumor samples HPV+ vs HPV-. Of 10,430 genes, 29 genes showed statistically significant differences (|logFC| > 1, fdr < 0.1) between the two groups (Fig. 1A). We performed a principal component analysis to assess the distribution of samples on a bidimensional space (Fig. 1B). Similarly, we investigated differential gene expression profiles in HPV- tumor samples vs HPV- normal samples. 1847transcripts were identified with a |logFC| > 1 and fdr < 0.1 (Fig. 1C). Contiguous spatial expression analysis of the 10th percentile of genes in HPV- tissues were carried out. 87 transcripts were overexpressed through the 3 stages, whilst 13 genes showed underexpression profiles.

Conclusions. Trascriptome sequencing represent a powerful tool in modern pathology, investiganting the alterations of gene expression in the transition from normal epithelium to invasive neoplasia can shed light in the pathogenesis and identify new molecular targets for an individualized therapy.

QUANTITATIVE IMAGE ANALYSIS OF PROLIFERATION AND MICROVESSELS DENSITY IN A MOUSE XENOGRAFT MODEL OF BREAST CANCER

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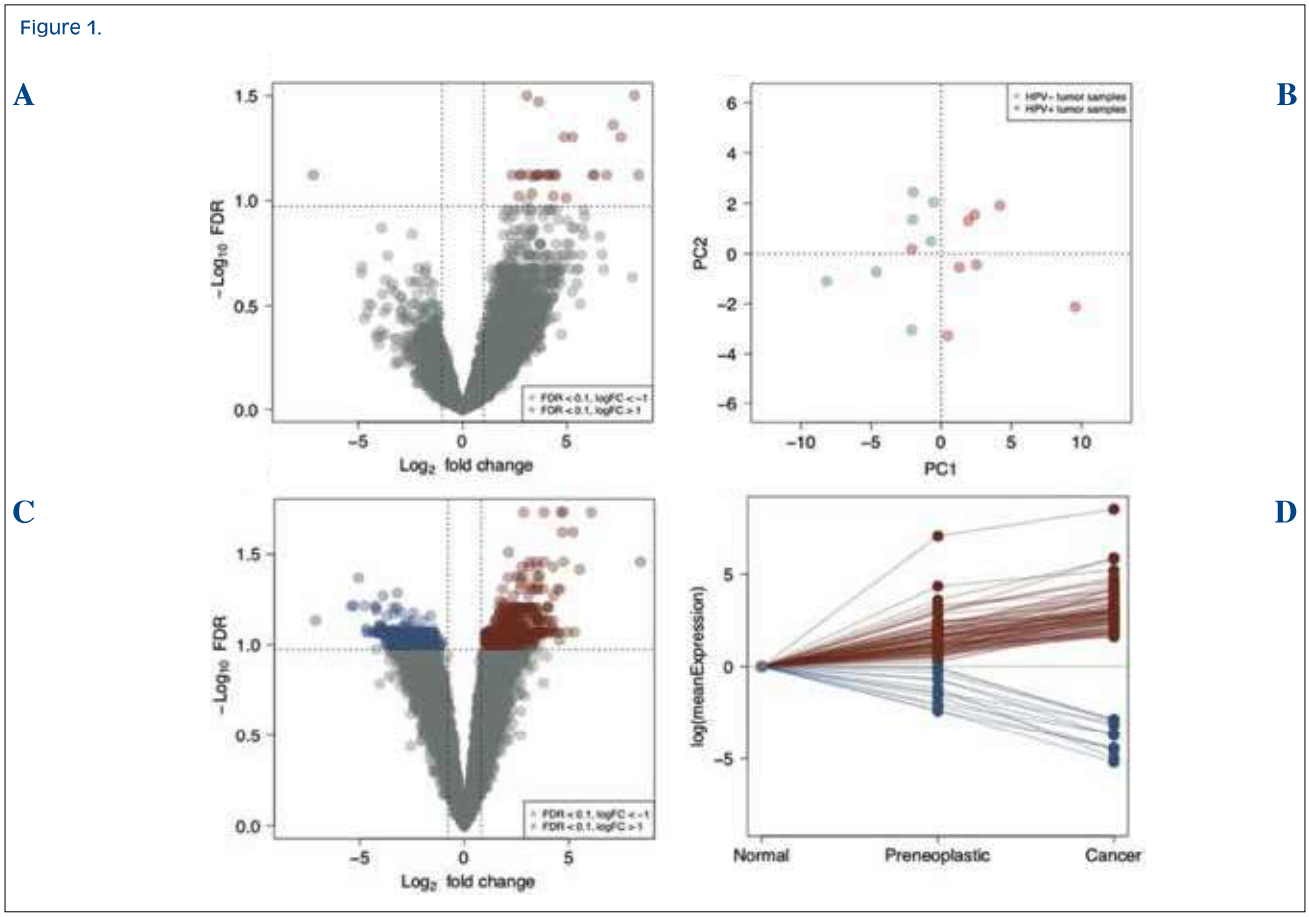
Objective. Proliferation index and tumor angiogenesis are considered prognostic factors in many malignancies. However, their quantification by immunohistochemistry (IHC) is not completely objective, being in part operator-dependent. We evaluated the proliferation index and microvascular density (MVD) by IHC in a mouse xenograft model of breast cancer and we quantified them using an image analysis software. Moreover, we focused on IHC changes after different treatment protocols.

Methods. The mice were allocated to six cohorts with different treatment protocols (controls, A, B, C, A+C, B+C). Twenty samples of breast cancer were histologically analyzed. We considered histological features as grading, necrosis, fibrosis, Tumor Infiltrating Lymphocytes (TIL), mitotic index (n° mitoses/10HPF), apoptotic cells (n° apoptotic cells/10HPF), and immunohistochemical markers for proliferation index (Ki67) and microvascular density (CD31 and CD105). IHC staining has been quantified by image analysis software Fiji-ImageJ®. After the selection of regions of interest (ROI), digital photographs of 4080x3072 Pixels^2 x 600 were taken and saved in Tiff format. The proliferation index with Ki67 was evaluated by manual counting, percentage of area (%A) and

Figure 1.



percentage of cells with semi-automatic counting (%C). MDV (CD31+ and CD105+) was evaluated as %A. All data were entered in a Microsoft Excel© spreadsheet and for statistical computation MedCalc© program was used.
Results. Quantitative image analysis proved to be reliable. The semi-automatic (%C) counting of Ki67 has shown an high correlation coefficient (Pearson correlation coefficient r=0,8; p<0,0001) when compared to manual counting, confirming its reliability other than its high grade of automation. MDV (CD105+) showed better results than MDV (CD31+) because of the cross- reaction with inflammatory cells in the latter. Regarding the Ki67 index, a decreasing and a paradoxical increasing were observed respectively after the treatment with B+C and B. Regarding MDV quantification (CD105+), a decreasing and a paradoxical increasing were seen respectively after the treatment with C+A and B, whereas MDV (CD31+) showed only heterogeneous results.
Conclusions. Quantitative image analysis allows to extract a numerical value from IHC assays, in an objective and reproducible manner, providing its possible utility in routine lab set. In particular, the %C resulted more rapid and automated than the other methods, reducing the operator-related confounding factors. Moreover, it helped us in revealing how some treatments -in our study- may reduce the proliferation index and tumor angiogenesis in breast cancer.

QUALITÀ E SICUREZZA

ROLE OF CYTOTECHNICIAN AT THE PASSAGE TO HPV TEST IN THE CYTOLOGY- SCREENING UNIT OF ASL BARI

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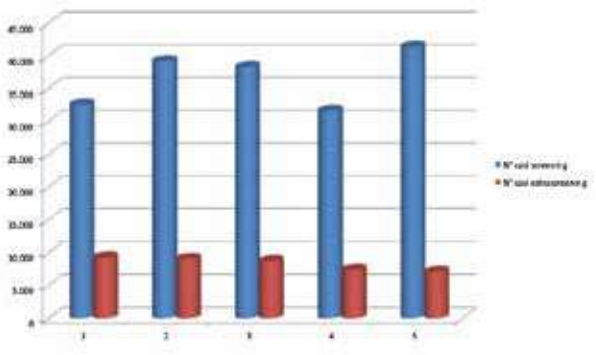
Background. Cervical cancer screening has traditionally been based on cervical cytology. Given the aetiological relationship between human papillomavirus (HPV) infection and cervical carcinogenesis, HPV testing has been proposed as an alternative to Pap-test [1 2]. The aim of this study is to define the cytotechnicians role in the cervical cancer screening at the passage to HPV test in ASL Bari.
Methods. The cytotechnicians a Biomedical Laboratory Professional with a scientific literacy in cytology and clinical skills to screen samples (he should not confuse true-negative results with false-negative results). He evaluates specimens and examines cellular samples under a microscope, looking for the presence of abnormal cells.Then he marks them and makes a preliminary diagnosis (Fig. 1).
Results. In the last five years the performances of Cytology and Screening Unit of ASL Bari were of 224.543 Pap test (Fig. 2, Tab. I). The cytotechnicians examined negative samples and controlled each phase (from the first analysis to the final evaluation of specimens). They worked under the direction of a pathologist who evaluated only the apparently positive samples [3]. The cytotechnicians carried out the analytical cycle with a workload range from 6 to 10 slide/hour (i.e. 36-60/day or 7,500/year) if the duty was limited to the screen only [4], but unfortunately the biggest problem of the Unit was the delay of TAT (turn around time) due to the chronic shortage of staff.

Figure 1. Work cycle of the cytotechnician.

Figure 2. Cytology Unit Performances in last five years.



Tab. I. Cytology Unit Performances in last five years.

Year	Pap test screening	Pap test extrascreening	Total
2013	32.546	9.276	41.822
2014	39.167	9.049	48.216
2015	38.282	8.746	47.028
2016	31.608	7.404	39.012
2017	41.373	7.092	48.465

Discussion. The new screening algorithm with HPV should reduce cytotechnicians workload because only women 25 to 34 years of age should be screened every three years with cytology alone (Fig. 3), while women 35 to 64 years of age should be screened every five years with HPV onlytesting (Fig. 4).
Conclusions. In our opinion the reduction of Pap test's number in the era of HPV testing will be an advantage for our Unit because it will allow us to provide a good quality control, such as inclusion of rapid prescreening and/or 100% rapid review of the cervical cytology examination, reducing false-negative results of routine screening. Consequently, cytotechnicians lowest workload will improve the diagnostic sensitivity and the reporting times will be shortened at the same time.