Endoscopy remission rate was very similar to the second histological score (36 children). No significant contribution came from the application of immunohistochemistry.
The comparison between the first work and this extension reveals a higher percentage of MH reached by only activity examination then by the architectural and inflammatory score (68% vs 53%) and a very high concordance with endoscopy.
Thirty-one patients were re-biopsed between 12 and 24 months after this therapy: among them 11 patients had disease relapse. Agreement between MH and follow up was found in 18 patients for the first score and 21 patients for the activity score (respectively 58% and 67% of cases). Relapses were predicted in 6 cases by the first score and 5 cases by the second; when the scores were summed, prediction was found in 7 patients (64%).
Conclusions. In this work, we explored the contribution of pathology in the evaluation of MH as an endpoint of a Azathioprine therapy in children affected by IBD.
At the beginning of this investigation, no MH definition existed in histopathology, and a good correlation with endoscopic MH was the goal of our investigation in MH.
Recent experiences converged to a simple MH based on absence of activity alone.
Our study follows this trend: when MH was defined just by the absence of activity in biopsies, correlation with clinical data and endoscopy was high. Moreover, when compared with follow up, about 2/3 of courses were predictable by MH.
MH based on evaluation of architectural changes and inflammatory infiltrate, tissue eosinophils was found less informative.
Prediction of relapse is probably the most interesting datum achievable by MH. The weak higher significance of the first score could indicate a contribution yet to be delineated from the whole evaluation of the architectural changes and inflammatory infiltrate in predicting relapse.

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IMMUNOGENIC ROLE OF NECROSIS-INDUCING COMPLEX RIPK1-RIPK3- MLKL-P IN HEPATOCELLULAR CARCINOMA

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Objectives. Necroptosis (NCP) is a form of programmed cell death characterized by the lysis of cellular elements and it involves the formation of a necrosis-inducing complex consisting of the receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3) and the mixed-lineage kinase domain-like protein (MLKL) (1). NCP is involved in promoting inflammation under pathological conditions and recent studies on NCP have shown that some cancer cells may show a preferential reduction in the expression of the key mediators of NCP, suggesting that it may negatively regulate tumorigenesis. When NCP occurs in cancer cells, dispersed elements in the tumor microenvironment may contribute to cancer development by influencing local immune response, which can be assessed by the quantification of T cell infiltration in cancer. This can be done by using a reliable and reproducible method as the immunoscore (2), that may be applied also to hepatocellular carcinoma (HCC) (3). Aim of this study was to assess expression of RIPK1, RIPK3 and phosphorylated MLKL in cohort HCC patients and their correlation with T cell infiltration and clinical follow-up data.
Methods. RIPK1, RIPK3 and MLKL-p expressions were assessed by immunohistochemistry in 76 FFPE samples of resected HCC patients. Expression was evaluated on a 4-tired scale based on the percentage of positivity stain in tumor cells, where 0 indicates absent staining, 1+ indicates less than 5%, 2+ indicates positive staining between 6% to 50%, and 3+ indicates positive staining >50%. Both overall and disease-free survival analyses were performed on the patients stratified by high and low expression of the necrosis-inducing complex. Specifically, patients showing 3+ staining in at least two genes of the necrosis-inducing complex were grouped separately from the other cases. Tumor and peritumoral infiltrating CD3 and CD8 T assessment on fully digitized HCC sections and molecular quantification of RIPK1, RIPK3 and MLKL-p are in progress.
Results. RIPK1 showed 0+ in 18/76 cases; 1+ in 27/76 cases; 2+ in 20/76 cases and 3+ in 11/76 with cytoplasmic localization or in the membrane. RIPK3 immunoprofile resulted in 13/76, 10/76, 11/76 and 42/76 positive cases for 0+,1+,2+ and 3+ respectively; with cytoplasmic localization. MLKL-p resulted in 35/76, 24/76, 13/76 and 4/76 positive cases respectively 0+, 1+, 2+ and 3+; with cytoplasmic localization or in the membrane (Fig. 1). Both overall and disease-free survival analyses showed significant correlation between poor prognosis and down-regulation of the necrosis-inducing complex, with log- rank test p-values 0.048 and 0.012, respectively.
Conclusions. Considering our preliminary data NCP occurs in a subset of HCC patients and it could represent an easy and reliable parameter for the evaluation of HCC immunogenicity and for the evaluation of tumoral aggressiveness. These results will be compared to those obtained in larger cohorts from publicly available datasets (e.g. data from TCGA and ICGC consortia) to validate the role of NCP in the hepatocellular carcinoma.

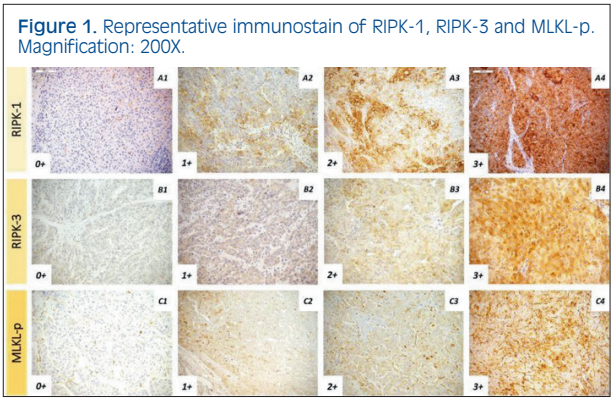

Figure 1. Representative immunostain of RIPK-1, RIPK-3 and MLKL-p. Magnification: 200X.

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THE MOLECULAR LANDSCAPE OF EXTRANODAL EXTENSION OF LYMPH NODE METASTASIS IN COLORECTAL CANCER

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Background. ExtraNodal Extension (ENE) of lymph node (LN) metastasis has recently asserted itself as a significant prognostic factor in colorectal cancer (CRC). The molecular background on the development of ENE phenotype has not been investigated, as yet.

Methods. A retrospective and consecutive series of 22 CRCs and their matched ENE- lymph node metastasis was enrolled (M/F=15/7; median age 70.2±13.7 years; grading G2=4, G3=18; staging IIIb=2, IIIc=14, IV=6; all Caucasians). Three different specimens per each case were obtained respectively from the formalin-fixed paraffin-embedded (FFPE) primary CRC, an ENE-negative LN metastasis and an ENE-positive one. DNA was extracted from 15 cases after neoplastic cell enrichment and, subsequently, it was profiled by the application of an hotspot multigene mutational custom panel, including 164 hotspot regions of AKT1, APC, BRAF, CTNNB1, KIT, KRAS, NRAS, PDGFRA, PIK3CA, PTEN and TP53 genes (Diatech Pharmacogenetics, Jesi, Italy) and run on a MassARRAY Dx Analyzer 4 (Agena Bioscience, Hamburg, Germany). Somatic mutations were corroborated by Sanger sequencing. The 66 selected specimens were analysed by immunohistochemistry for PDL-1 (clone 22C3; Dako, Glostrup, Denmark), CD4, CD8, CD68 and CD80. The percentage of cells showing a positive immunoreactivity was appraised in: I) core of CRC; II) invasive front of CRC; III) core of neoplastic cells in both the ENE-negative and ENE-positive LN metastasis; IV) invasive front of the ENE-positive LN metastasis.

RESULTS: 14 out of 15 CRCs (93%) showed at least a driver mutation in the primary tumour. The most frequently mutated gene was TP53 (8 tumours), followed by APC (6), BRAF (4), KRAS (2), NRAS (2) and PIK3CA (2). In 11 out of 15 CRCs (73%) the mutational profiling of the primary tumour was consistent with what obtained from the two matched metastatic LN (ENE-negative and ENE-positive). The molecular inconsistency of the last 4 cases could be referred to the intratumour heterogeneity in accordance with Literature. A significantly higher percentage of CD4, CD8 and CD68 positive cells was observed at the invasive front of CRCs and their matched ENE-positive LN metastasis in contrast with what observed at the core of both CRCs and neoplastic cells in their matched ENE-negative and ENE-positive LN metastasis. A significantly higher CD80 percentage was solely observed at the invasive front of ENE. No significant difference of PDL-1 positive cell percentage was observed among the different specimens.

Conclusions. The neoplastic ability to infiltrate the LN capsule reflects the aggressiveness of the primary tumour but, in our study, it seems there is no ENE specific molecular signature. However, the detection of a significantly higher percentage of CD4, CD8, CD68 and CD80 cells at the ENE invasive front might encourage further investigations on a possible application of immunotherapy in ENE-positive CRCs.

EVALUATION OF ULTRARAPID UREASE TEST FOR HELICOBACTER PYLORI IDENTIFICATION

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Background. Accurate detection of Helicobacter pylori (HP) is essential for managing infected patients and for eradicating therapy. Since the discovery of HP, several diagnostic methods have been developed with the aim of accurately detecting of this bacterium. These tests include non invasive methodserology, urea breath test, or stool antigen test and invasive methods, such as, culture, histological examination, and rapid urease test, which require upper gastrointestinal endoscopy to obtain gastric biopsy samples. Among these, histological examination is one of the most useful diagnostic tests for HP infection. Recently, it has been produced a new era of urease test (ultrarapid) enables a rapid diagnosis within 5 minutes during gastroscopy. We compare this test with histological and immunoistochemical (IHC) examination. For all biopsies we evaluate the histological quality after its utilisation.

Methods. We start with the observational prospectical monocentric study (PRIMUM) enrolling 42 patients >18 years in order to evaluate the efficacy of the ultrafast test (BioTest HD®). Criteria of exclusions were a suspicious of carcinoma and a previous utilisation of proton pump inhibitors, antibiotics or colloidal bismuth by patients. The antral gastric biopies were immediately immersed in the liquid of the test comprehensive of blue of bromotimolo and urea (ph5.5). After 5 minutes the gastroenterologists evaluated the results of test according a scale from 0 to 3. In the positive cases the colour of test changed from yellow (Fig. 1A) to blue (Fig. 1B). After this evaluation biopsies were fixed in formalin and sended to the routinely process. The pathologists evaluated the quality of specimen (antral and body biopsies), the inflamamtory lesions and the presence of HP infection both with Ematoxilin&Eosin and Giemsa stained slide and by immunoistochemistry (IHC).

Results: Between 42 cases 10 (23,8%) resulted positive to