Local and systemic pleuric -peritoneal and biohumoral changes and risk of post-surgical environmental contamination in children (0 -16 years) undergoing minimally invasive surgery

Brief description of the proposal

CO2-insufflation during minimally invise procedures causes distension of the abdominal and thoracic cavity. Mechanical and biochemical stimuli can modify the peritoneal and pleural environment and promote inflammatory infiltration. Given the widespread use of minimally invasive techniques even in children, it is important to verify whether the CO2-insufflation induces local morphological changes of the peritoneum and pleura, and systemic alterations. The study of serosa cell infiltrates and the identification of local and systemic mediators that regulate inflammatory cells may be responsible for the clinical symptoms reported by patients undergoing this technique, and therefore their prevention could represent an important means of treatment. Furthermore, evaluating the possible environmental contamination after CO2 evacuation is important for the safety of components of the operating room. Based on this background we aim to: 1) investigate the impact of MIS on human mesothelium in terms of inflammatory response, cellular changes, and oxidative stress; 2) verify the systemic changes in cytokines levels after MIS in childhood; 3) evaluate difference between clear and dirty MIS in environmental contamination; 4) identify possible cross-infection from patients to healthcare workers.

State of the art

Minimally invasive surgery (MIS), such as laparoscopy and thoracoscopy, in children has been widely adopted in the treatment of many pathologies and has given very satisfactory results. The main advantages obtained by this technique are the reduced hospitalization, the reduction of postoperative pain, better aesthetic results, and early recovery with quick return to daily activities. However, some patients complain of pain, gastrointestinal discomfort, and peritonism which generally is over after about 24 hours. The abdominal cavity is characterized by a normal physiological state with biochemical and physical conditions of 0/3mmHg of intra-abdominal pressure (IAP), whereas intrathoracic pressure is -2/-6mmHg. Carbon-dioxide (CO2) insufflation, which is required for MIS, can change the serosa environment through both physical and chemical interactions. An increased pressure decreases perfusion of the operating area. Due to hypoperfusion and ischemia-reperfusion injury, reactive oxygen radicals and inflammatory cytokines accumulate(1–2). This injury depends on the magnitude and length of the insufflation, however there is not enough information on the complications due to increased pressure. Studies performed on animals had confirmed the systemic oxidative stress caused by high IAP. The redox status of the body is tightly regulated and returns to normal within 24 hours after surgery, as shown in the majority of the studies. In particular, in one study involving children, there was no change in markers of oxidative stress after either open or laparoscopic surgery(3). Gutt and Schmandra observed that by increasing the IAP, blood flow will decrease and at 12mmHg it becomes minimal. Hypoxia will evolve and abdominal organs will be damaged by reactive oxygen species(4). Polat et al. observed that MDA and sulfhydryl-group concentration had increased if they applied higher IAP (10 vs. 15mmHg) during cholecystectomy in children(5). The gas used universally to induce pneumoperitoneum (PN) is CO2. When CO2 is insufflated, it reacts with H2O from the peritoneal fluid, reduces the peritoneal pH and creates an acidic environment that has been seen to modify local defense mechanisms(1-4) and inflammatory response (5). CO2 insufflation has been shown to reduce the inflammatory peritoneal response compared to conventional laparotomy and the use of air room. The serosa consists of a single continuous layer of mesothelial cells that are located on a basement membrane, under which there is a layer of fibrous connective tissue. Mesothelial functions include: selective fluid and cell transport(4;10;8); physiological barrier(1;2;4;8); immune induction, modulation, and inhibition (8;10); tissue repair and scarring (9;10); preventing adhesion and tumoral dissemination (3). The creation of a PN has been shown to induce structural changes in the mesothelial surface layer, which are visible under the microscope. It has been shown in rats that, 24 hours after the induction of a PN of 12mmHg, there is peritoneal infiltration of eosinophils, granulocytes, and mast cells (6). Volz et al (7) showed characteristic

ultrastructural changes in the peritoneal surface after CO2 insufflation: mesothelial cells were swollen with widening of the intercellular spaces and infiltration of macrophages. Previous papers have reported that chemical, physical, and biological effects of CO2 on peritoneum causes inflammation and tissue modifications(6,8,9). The inflammatory response is characterized by eosinophils, granulocytes, mastocytes, macrophages, and lymphocytes tissue infiltration, cells involved in chronic inflammation. These alterations seem to be mainly related to the pressure and acidic environment induced by the gas insufflation(6,8). Furthermore, PN modifies serum and peritoneal concentrations and tissue expression of the chemokines CCL5 and CCL2. Previous findings reported that RANTES/CCL5 showed the most dramatic increase(9). In the era of MIS, knowledge of the basic physiological mechanisms that are modified by gas insufflation is essential in making this surgical approach even less invasive. Beside the patient safety, also the risk for healthcare workers (HCWs) represents a problem of huge importance due to contact with biological materials, possible environmental pollution, manipulation of instruments, objects and potentially contaminated materials(1). Insufflation and desufflation during MIS may also aerosolize and distribute biological material(27) and harmful substances due to electrocautery smoke and plumes from energy devices. Whilst there is currently no evidence that desufflated CO2 or surgical smoke plumes present a significant infectious risk, there is not a wealth of literature to inform current practice and no studies are available for children. Therefore a further aim of this study is to investigate the safety of laparoscopy for HCWs.

Detailed description of the project: methodologies, objectives and results that the project aims to achieve and its interest for the advancement of knowledge, as well as methods of dissemination of the results knowledge, as well as methods of dissemination of the results achieved

As reported above, laparoscopy and thoracoscopy can compromise mesothelial integrity and biology, therefore gaining insights in the pathophysiological mechanisms modified by these techniques is crucial in making this surgical approach much less invasive. The mesothelium maintains an extraordinary balance between all of its components and factors, such as chemokines, cytokines which play an important role in the activation and recruitment of leukocytes to inflammatory sites, but the knowledge of these mechanisms is the real challenge in minimally invasive surgery (MIS). Animal experiments have suggested that gas insufflation might adversely affect the serosa environment. Moreover, MIS constitutes a risk procedure for HCWs, as it exposes them to biological risk through the contact with biological materials, possible environmental pollution (airborne and surfaces), manipulation of instruments, and objects. Air cleanliness is crucial for protection of patients from surgical site infection and of healthcare workers and it must be preserved by controlling air contamination.

Based on this knowledge, the present experimental study aims to:

1) investigate the impact of MIS on human mesothelium in terms of inflammatory response, cellular changes, and oxidative stress;

2) verify the systemic changes in cytokines levels after MIS in childhood;

3) evaluate difference between clear and dirty MIS in environmental contamination;

4) identify possible cross-infection from patients to healthcare workers.

Methods Inclusion/exclusion criteria

Patients undergoing elective thoracic or abdominal surgery aged 0-16 years will be enrolled. Patients affected by immunological defects, genetic syndromes, and acute or chronic inflammatory diseases will be excluded. Patients will be stratified according to age into two groups: infants (age 10 dots per cell with less than 10% positive cells having dot clusters (visible at 20x magnification); score 4, >10 dots per cell with more than 10% positive cells having dot clusters (visible at 20x magnification). Study of cellular damage

from oxidative stress Lipid peroxidation marker malondialdehyde (MDA) concentration, endogenous antioxidant reduced glutathione (GSH) and sulfhydryl-group (SH-) concentrations, antioxidant superoxide-dismutase (SOD) and catalase (KAT) activities will be measured from whole blood for detecting the magnitude of oxidative stress. A mixture of 4.5 ml TBA (thiobarbituric acid) and TCA (trichloroacetic acid) will be added to 0.5 ml plasma or diluted blood. Samples will be incubated for 20 minutes at 100°C then cooled at 0°C. Blood will be centrifuged in a cooled centrifuge at 4000 rpm for 15 min. The concentration of MDA will be determined using a spectrophotometer at 532 nm and expressed in nM/mle. For the determination of GSH and SH- a mixture of one ml quintuple blood sample and 4 ml trichloroacetic acid (TCA) will be used. The mixture will be centrifuged at 4000 rpm for 15 min. The supernatant will be added to 4 ml TRIS buffer (0,4 M, pH:8,7) 2 ml and 100 _l DTNB (5.5'-ditio-bis-2-nitro-benzoe acid) will be added to the mixture immediately before measurement. The concentrations of GSH and SH- will be determined using a spectrophotometer at 412 nm and expressed in nM/ml. For the determination of SOD activity 1 ml blood will be mixed with EDTA, then 9 ml Hartman's solution will be added to the blood sample. The mixture will be centrifuged at 2000 rpm for 5 min. After discarding the supernatant, the washing procedure will be repeated. A mixture (2: 1) of 1 ml chloroform and ethanol will be added to 1 ml haemolysed red blood cells and they will be centrifuged at 17000 rpm for 4 min. Supernatant will be separated thereafter, and adrenalin (16.488 mg adrenalin diluted in 10 ml 0.1N hydrochloric acid) will be added to it. The concentration of SOD will be determined by using a spectrophotometer at 480 nm and expressed in U/ml. For the determination of MPO activity 1 ml work solution (10.9 ml Na-citrate, 100_l o-Dianisidin) will be mixed with 200_l plasma. The compound will be incubated at 37 C for 5 min, then 1 ml of 35% perchloric acid will be added to the mixture and centrifuged at 2500 rpm for 10 min. MPO concentration will be measured using spectrophotometer at 560 nm and expressed in U/l. To determinate the catalase enzyme level we will mix 2 ml of buffer, 1 ml of peroxide solution and 100 times diluted, washed red blood cell. With spectrophotometer we will measure the loss of peroxides at 240 nm. Catalase levels will be expressed in BE/ml. Ultrastructural study of peritoneum by electron microscopy and immunocytochemistry Tissue specimens will be fixed with Karnovski for 3h at 4°C and post-fixed in 1% OS04 in O.1 M cacodylate buffer pH 7.35, dehydrated in ethanol and embedded in Araldite. Ultrathin sections obtained with a diamond knife will be stained with uranyl acetate and lead citrate. Immunohistochemistry Tissue specimens will be fixed with 4% paraformaldehyde and 0,1% glutaraldehyde for 3h at 4°C, dehydrated in ethanol and embedded in London Resin White. Ultrathin sections obtained with a diamond knife will be stained with uranyl acetate and lead citrate and incubated with primary antibodies anti IL-33 and IL-18. The reaction will be revealed with secondary antibodies gold- labelled. All samples will be observed under Philips CM-10 transmission electron microscope (Huntsville, Alabama, USA) and the images will be acquired with VELETA EMSIS camera. Study of microbiological contamination Microbiological samplings will be carried out on both clean (control) and dirty MIS (case) interventions in order to investigate the impact of the two types of intervention on environmental contamination; for this purpose, all the possible factors that influence the microbial load present in the operating theatres (OTs) will be collected: number and identification of healthcare workers present in the OTs for every surgical intervention, correct use of protective personal equipment (PPE), status and type of Heating, ventilation and air conditioning (HVAC) system with switch-on time, number of door openings and related reasons and environmental thermo-hygrometric parameters. On the other hand, microbial contamination by patients will be investigate through a swab that will be collected at the beginning of surgical operations for clean ones and trough a sampling of peritoneal fluid for dirty ones. These samples will be tested for detections of pathogen microorganism, trough methods described below also for environmental investigation. In presence of environment microbial contamination by pathogen microorganism HCWs that were present in OT will be followed through ad an hoc telephone survey for the onset of the specific disease for a period corresponding to the incubation time of the single detected pathogen and other specified sources of infectiousness and confounding factors will be investigate. Environmental investigation should be performed both on surfaces that on air sample. Here below we briefly describe the sampling methods. Surfaces The microbiological samplings for the definition

of the level and type of surface contamination will be carried out in the measure of number one (1) sampling for surgery for the "at rest" theatre and one (1) sampling for the "operational" theatre (at the end of the intervention). Sampling method Contact method: it will be used for flat surfaces (UNI EN ISO 14698-1 standard), using culture media suitable for the microorganisms sought and the accessible contact surface must be over 20 cm2; for this purpose, 24 cm2 Replicate Organism Direct Agar Contact (Rodac) plates will be used. The plates used will be the following: a) Plate Count Agar for the total count of microorganisms b) selective plates in order to identify the following potential pathogens: Vogel-Johnson Agar for Staphylococcus spp, Cetrimide Agar for Pseudomonas spp, Rose Bengal-CAF for yeasts, Violet Red Bile Glucose Agar (VRBG agar) for Enterobacteriaceae and Bile-Esculin Agar for Enterococcus spp and Clostridium difficile Agar for Clostridium difficile. The convex part of the RODAC plate will be applied to the surface to be monitored with a contact time on the surface of 30 seconds by applying a uniform and constant pressure to the entire area (to allow a better standardization of the method, specific applicators can be used). The sampling will involve 3 points per OT both in the "at rest" and "operational" ones: operating bed, scialytic and overhead table. "At rest" samples must be taken on dry surfaces, after the end of the room sanitization operations and after it has been closed and empty for at least 30-60 minutes (this time is considered sufficient for the action of the disinfectants). This scheme involves a number of Rodac plates equal to: N = (n° interventionsx1x7) for withdrawals in the "at rest" OT + (n ° interventionsx1x7) "operational" OT ex. for 50 interventions: (50x1x7) + (50x1x7) = 700 plates To this must be added the plates for the verification of sterility (equal to n operations). In fact, this scheme involves a number of Rodac plates equal to: N = (n ° interventionsx1x 7) for withdrawals in the "at rest" room + (n ° interventionsx1x7) "operational" room The transport of the RODAC plates, after sampling, will take place by keeping the plates at a temperature between 15 ° C - 20 ° C. The time between sampling and incubation in the thermostat must be as short as possible, and in any case no later than 12 hours, using an appropriate portable system for temperature control. Incubation must be carried out respecting the following values: • at 37 ° C for 48/72 hours to evaluate bacterial growth; • at 25 ° C for another 24/48 hours to check the growth of Candida spp. yeasts. At the end of the incubation period, we count the colonies that have developed on the surface of the RODAC plate, (24-48 hours for the total count, 48 hours for the count of the various microorganisms excluding the fungi, 72 hours the fungi). Samples will be classified as positive according to the manufacturer's instructions for Rodac contact plates (> 14 colonies corresponding to 117 CFU / 100 cm2). Each plate will be photographed after development and archived, in order to allow the comparison, even in the future. Growth of C. difficile will be performed with 5 BD Clostridium Difficile Agar with 7% Sheep Blood or Blood Agar plates and verification will be performed with Latex test (eg Ref. 96144 Latex kit Clostridium Difficile-Liofilchem) Subsequently, automated biochemical methods (VITEK® 2, Bio-Mérieux, France) will be used to identify other microorganisms grown in subcultures and to assess their susceptibility to antimicrobials. Method with swab: it will be used to search for germs on flat and non-flat surfaces, and especially in areas that are difficult to reach with the plates. This is a predominantly qualitative or semi-quantitative analysis. The swabs will be used inside a sterile disposable mask (10x10 cm2) which will be placed on the analyzed surface. The swab must be previously humidified with sterile isotonic solution and must then be passed in parallel and perpendicular strips by rotating it in the various directions. Swabs will be immediately placed in a 5 ml tube containing enrichment broth and only subsequently incubated at 37 ° C for 24 h. Ministero dell'Università e della Ricerca MUR - BANDO 2020 We set up subcultures in selective agar culture media for positive samples: Mannitol Salt Agar for isolation and identification of Staphylococcus spp; MacConkey Agar for Gram-negative bacteria; Enterococcosel Agar for Enterococcus spp; Cetrimide Agar for Pseudomonas aeruginosa, Sabouraud agar for yeasts and molds. Subsequently, automated biochemical methods (VITEK® 2, Bio-Mérieux, France) will be used to identify microorganisms grown in subcultures and to assess their susceptibility to antimicrobials agents. Air Samples to evaluate air microbial contamination r (expressed as CFU / m3) will be collected from the center of the room, using a semi-automatic sampler (SAS Super100, Sampler Air System, PBI), which draws a volume of 180 l / min. The SAS will contain a 55 mm diameter plate containing the different selective and non-selective agar culture

media (PCA, Mannitol Salt Agar, MacConkey Agar, Enterococcosel Agar, Cetrimide Agar, Sabouraud agar and Clostridium difficile elective agar). The operator must wear appropriate operating room clothing, wash his hands and wear gloves and, during the collection, the operator must remain outside the operating room. Sampling should be carried out in the center of the operating room (at the operating table level) at approximately 1.5 meters from the floor and within 1 meter from surgical wounds. Sampling will be start at the beginning of all surgical operations by taking the first sample at the stage of the surgical incision. The subsequent samples (minimum of 4 samples) were taken in the next phases every 5 to 10 minutes depending on the duration of the intervention with a sample at the end of the surgical operation. At the same time, the following parameters will be recorded: number of healthcare workers present in the room and correct use of PPE, status and type of HAVC with switch-on time, number of door openings and related reasons and environmental thermo-hygrometric parameters. This scheme involves a number of RODAC plates equal to a minimum of: N = (n ° interventionsx1x 7) for withdrawals in the "at rest" OT + (n ° interventionsx4x7) operational OT e.g. for 50 interventions: (50x1x7) + (50x4x7) = 1750 plates Samples will be classified as positive according to ISPESL guidelines but the number of colony forming units will be determined. In fact, this scheme involves a number of Rodac plates equal to a minimum of: N = (n ° interventionsx1x 7) for withdrawals in the "at rest" room + (n ° interventionsx4x7) operational hall e.g. for 50 interventions: (50x1x7) + (50x4x7) = 1750 plates Samples were considered positive according to the parameters suggested by the “guidelines on standards of safety and occupational health in the operating department” of the Italian Agency for prevention, occupational health and safety (ISPESL) which admits for OTs “at rest” an air contamination limit 35 cfu/ m3 and for OTs “in use” values 180 cfu/m3 (turbulent flow) and 20 cfu/m3 (laminar airflow). English National Health Service - Health Technical Memorandum 2025 standard However, since the purpose of these samplings is to evaluate the correct functioning of the HAVC system and the disinfection procedures, as indicated by the ISPESL guidelines, we will determine also the following values: 1) the indicative values of the correct functioning of each operating room (Target values) 2) the values that indicate operation within the limits of acceptability (Alert values) 3) the values that highlight the need for an intervention as they show that we have moved away from the qualitative standard of correct management (Action values). In fact, this scheme involves a number of Rodac plates equal to a minimum of: N = (n ° interventionsx1x 7) for withdrawals in the "at rest" room + (n ° interventionsx4x7) operational hall e.g. for 50 interventions: (50x1x7) + (50x4x7) = 1750 plates The English National Health Service-Health Technical Memorandum 2025 standard provides for biological contamination in the ambient air, near the operating table, for conventional operating rooms in rest conditions, with turbulent flow VCCC system the value 35 CFU/m3. It is possible to reach contamination levels much lower than the indicated value. However, since the ultimate purpose of these samplings is to evaluate the correct functioning of the VCCC system and the disinfection procedures, as indicated by the ISPESL guidelines, the following will be determined: 1) the indicative values of the correct functioning of each operating room (Target values) 2) the values that indicate operation within the limits of acceptability (Alert values) The values that allow to evaluate the microbiological quality "at rest" of the operating room will be determined by means of a diagram of the results obtained (at least 20 values) having on the abscissa the UFC / m3 value and on the ordinate the number of samples; first of this a complete technical check of the room will be carried out in order to ensure that there are no initial malfunctions that could offset the measurements (control of particulate matter, pressure, the number of air changes of the VCCC and the integrity of the filters) . The construction of these values will take place through the statistical analysis of the environmental data collected in which: • Target-Values: they are included between the mean and one standard deviation (SD) of the data collected. They are only acceptable in the absence of pathogenic germs (S. aureus, A. niger or A. fumigatus or Gram negative bacilli). • Alert values: they are included between a SD and the 95th percentile value of the observations obtained. • Action values: these are the values higher than the “alert” ones. Finally, we set up subcultures in selective agar culture media for positive samples: Mannitol Salt Agar for isolation and identification of Staphylococcus spp; MacConkey Agar for Gram-negative bacteria; Enterococcosel Agar for Enterococcus spp; Cetrimide Agar for Pseudomonas aeruginosa,

Sabouraud agar for yeasts and molds. Subsequently, automated biochemical methods (VITEK® 2, Bio-Mérieux, France) will be used to identify microorganisms grown in subcultures and to assess their susceptibility to antimicrobials agents. Statistical analysis Continuous variables will be checked for normal distribution and eventually log transformed to achieve normality. Continuous variables will be expressed as mean and standard deviations, categorical variable as percentage. Cohen K agreement test will be performed to test inter-observer agreement. Difference between groups will be assessed with Student t-test for matched pairs and independent samples as appropriate. A value of $P < 0.05$ will be considered statistically significant.

Sample size

A sample of 52 children and 52 infants will achieve the 80% power to detect an effect size of 0,40 with a Student t-test for matched pairs with an error of 0.05.

Ethics

Ethic Committee of University of Campania “Luigi Vanvitelli”, of Siena and Ancona has already approved the study protocol (n. 0007953/i, date 06/04/2020). Written informed consent from patients and parents will be obtained before enrollment. Results that the project aims to achieve and its interest for the advancement of knowledge MIS represent the most performed surgery in childhood thanks to better aesthetic results, sorter hospitalization, and good performance. However, it is not free from short and long-term complications. Even considering the literature produced on this topic, there are no studies available in childhood that can eventually confirm experimental findings. This project aims to contribute to this filed of research. In fact, the identification ofpathophysiological mechanisms underlying serosa environment changes might allow surgeons in modulating MIS features and pharmacotherapy to minimize tissue damage.

Dissemination of the results achieved

Project dissemination will follow three main strategies, in which communication contents and channels are tailored to different target groups: 1)traditional scientific presentations and manuscripts will be presented in scientific conferences and journals; 2)press and media coverage with traditional tools such as press releases, leaflets; 3)promotion of project activities through social networks.

Project development, with identification of the role of each research unit, with regards to related modalities of integration and collaboration

The present project will be developed according to the following objectives. Objective 1: to verify whether the CO2-insufflation induces local morphological changes of the serosa (peritoneum and pleura) and systemic biohumoral modifications in terms of inflammation and oxidative stress. Objective 2: a) to investigate the impact on environmental contamination (air and surface contamination) in clean and dirty laparoscopy interventions in order to determine the presence of statistically significant differences between the two types of intervention and b) to identify possible cross-infection from patients to healthcare workers Objective 1 All the units will contribute to the project with different tasks according to the clinical and research experience of the group. Four units have wide clinical experience in pediatric laparoscopy and thoracoscopic (Unibo, Unisi, Unime and Unicampania, in this unit there is also a researcher from University Politecnica delle Marche with clinical activity at a Pediatric Hospital Salesi). One Unit Unina has a broad and solid experience in studying pathological sampling with immunohistochemistry and electron microscopy). The main objective will be reached with the recruitment of the patients according to the methods reported, in particular Unit Unime, Unisa and Unicampania with UniMarche will treat with laparoscopy technique patients over 12 months of age, Unibo and the subgroup of UniMarche will treat and collect patients treated with laparoscopy or thoracoscopy below the age of 12 months and also over 12

months. All the studies on inflammatory response will be led by UniCampania laboratories, receiving samples from all the clinical units. The biohumoral samples for the evaluation of the celluar damage for oxidative stress will be conducted in the laboratories of Unisi receiving samples from all the clinical units. The study of peritoneal-pleuric immunoistochemical histological damage (samples from Unime, Unibo, Unisi and Unicampania) will be led by the unit of Unina. Objective 2 This study will be led in one operating room of the unit Unime to avoid any confounding error due to the different protocols of ventilation of the operating rooms, number of people attending the operation and other elements that could contribute to the contamination of the operating area. The following figure summarizes the role of each research unit, with regards to related modalities of integration and collaboration.

Possible application potentialities and scientific and/or technological and/or social and/or economic impact, with indications of the possible use of research infrastructures

Laparoscopy influences both peritoneal integrity and its biology, the immune system, and evokes peritoneal acidosis by CO2 insufflation. Furthermore, its influence on capillary circulation is responsible for oxidative stress. This experimental study aims to verify the local and systemic modifications induced by pneumoperitoneum evaluating these changes at well-known variables such as pressures and insufflation flow rates according to protocol and children age. Due to the wide spread of mini-invasive technique and laparoscopy in pediatric age, which has peculiar aspects for the different phases of somatic development, it is therefore important to determine which variable will interfere in the clinical symptomatology reported by patient subjected to this technique. To the best of our knowledge, this is the first study that investigates and validates the experimental findings about biological and histological modifications during and after laparoscopy in children. On the basis of our results, and by clarifying the mechanisms underlying peritoneal modifications after pneumoperitoneum it will be possible to modulate laparoscopic technique and post-surgical therapy with the aim of reducing tissue damage and post-operative complain. If we take into account that laparoscopy has become a widely used surgical approach in substitution of traditional laparotomic technique, we should make this technique even less Ministero dell'Università e della Ricerca MUR - BANDO 2020 invasive. In particular, the study of inflammatory mediators and their modulation with pharmacotherapy will be helpful in the management of post-operative pain and recovery with a final reduction of hospitalization length. Moreover, it has been reported that pneumoperitoneum alters abdominal circulation via reducing peritoneal capillary flow. The ischemia-reperfusion cycle increases the oxidative stress radical's production with tissue damage, mesothelial denudation, and possible adhesions formation. Therefore, efforts in reducing oxidative-stress damage might be useful in preventing long-term laparoscopy-related comorbidities. In addition, it should be highlighted that there are no previous studies investigating cellular and histopathological alterations in children. Finally, the study of the evacuation gas during laparoscopy might identify infectious agents contaminating abdominal environment. In the era of minimally invasive surgery, knowledge of the basic physiological mechanisms that are modified by laparoscopy and pneumoperitoneum is essential in making this surgical approach even less invasive. Furthermore, targeted therapy might be useful in lowering health costs in terms of hospitalization, drug prescription, and long-term complications. Moreover it must be taken in consideration that in Italy HCAIs (Healthcarecare Associated Infections)amount to about 450,000-700,000 per year, causing patients' death in over 1 percent of cases (1). The cost, mainly resulting from the increase in days of hospitalization, can range from € 4,000 for a patient hospitalized in a medical ward to € 28,000 for a patient in an intensive care unit (2). The clinical best care practices (CBPs) of hand hygiene, hygiene and sanitation, screening, and basic and additional precautions aim to reduce this burden and all studies demonstrated their cost effectiveness. The average yearly net cost savings from the CBPs ranged from $252,847 (2019 Canadian dollars) to $1,691,823, depending on the rate of discount (3% and 8%) and the average incremental benefit cost ratio of CBPs varied from 2.48 to 7.66 (3). Furthermore with the term of HAIs we must consider not only infections of patients but also infection of HCWs during the course of their work. In this case we must consider several factors:HCWs were frequently implicated as the source of nosocomial VPD transmission in

health care settings and they have frequent contact with high risk patients; in fact, they continue to work while sick or if they are asymptomatic can still be infectious before diagnosed. So investigation in this filed are important in order to reduce risk of outbreaks in health care facilities (together with the application of CBPs and active immunization), decrease staff illness and absenteeism and reduce costs resulting from loss of productivity. This study so could be important both on social that on economic terms to avoid further HCAIs and to investigate the potential role of future preventive measures to reduce spreading of microorganism from patients to HCWs. 1. Epicentro. Infezioni correlate all'assistenza. Available from: http://www.epicentro.iss.it/ problemi/infezioni_correlate/epid.asp 2. ASSOBIOMEDICA Federazione nazionale per le tecnologie biomediche, diagnostiche, apparecchiature medicali, dispositivi medici borderline, servizi e telemedicina La posizione associativa in tema di:infezioni ospedaliere. Giugno 2011. Available from: http://www.assobiomedica. it/static/upload/_con/controllo-delle-infezioniopsedaliere.pdf 3. E. Tchouaket Nguemeleu, I. Beogo, D. Sia, K. Kilpatrick, C. Séguin, A. Baillot, M. Jabbour, N. Parisien, S. Robins, S. Boivin. Economic analysis of healthcare-associated infection prevention and control interventions in medical and surgical units: systematic review using a discounting approach. Journal of Hospital Infection,Volume 106, Issue 1, 2020,Pages 134-154. https://doi.org/10.1016/j.jhin.2020.07.004.

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