Meconium - biomarker in ortho- and pathomorphogenesis algorhythm. Implications in medico-legal investigation

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Received: 13.01.2009/ Accepted: 20.02.2009

Abstract: Macro- and microscopic anatomic analysis of 84 fetus deceased ante partum, brought up the problem of meconium evaluation as a morphogenesis biomarker, starting with its distirbution in time and space at the level of intestinal sectors, and ending to the illicit drugs used by the mother in pregnancy.

Key words: drugs, meconium in utero exposure, meconium ileus, meconium plug syndrome

The physiochemical features of meconium, its temporo-spacial location and its implementation in orthology, pathology and medico legal toxicology, determined the elaboration of numerous projects concerning genesis, antenatal evolution of the structures and its account as a biomarker.

The aim of the paper is that the signification of the meconium stereotopography during the dynamic stages of ante partum ontogenesis should be clearly implemented into the pathology and medico-legal investigation pointing out its role as a biomarker.

The objectives of the paper were determined by the debates upon the problems existing in the normal and pathologic morphology such as: 1. What are the determining factors of the location and intestinal content volume formation in a fetus?; 2. What correlations exist between those parameters: gestation age and fetus antepartum anatomical evolution?; 3. Why ileum is the first intestinal sector storing meconium? 4. When and why the ante partum peristalsis appears?; 5. Could the spasticity of the distal colon by the circular muscles contraction influence the antepartum meconium stereo distribution?

MATERIALS AND METHODS
The study of meconium as a biomarker was performed by using a number of 84 fetuses aged between 13-28 gestation weeks.

Meconium volume and distribution were registered on intestinal sectors named on embryogenesis criteria. Meconium accumulation effects into the alimentary channels were evaluated by micro anatomic methods. Fragments removed from different sections were stained using Hematoxylin-Eosine, Van Gieson, Giemsa and Gömöri methods. Macro anatomic imagery was achieved by using a Canon camera, EOS 1ds Mark II. We used the research microscope Eclipse 600 equipped with a digital system for image recording to perform the examination of the micro anatomic sections.
RESULTS

The assessment of meconium stereo distribution and its transit effects through the digestive channels/canalis digestorius are possible by a morphologic double analysis: macro anatomical and micro anatomical ones.

Different problems created by the ambiguity of the terminology used to identify meconium location and formation mechanism determined us to work out an intestine sectorizing on an embryogenesis criterion. Small intestine (intestinum tenue) was split into three sectors such as: inframesocolic duodenum, jejunum and ileum.

Large intestine (intestinum crassum) was classified into the proximal colon, distal colon and rectum. We took into consideration three sectors in the “proximal colon”, to locate meconium: caecum, ascending colon/colon ascendens and transverse colon/colon transversum (2/3 right). Within the “distal colon” we localized meconium into four sectors such as: transverse colon/colon transversum (1/3 left), descending colon / colon descendens (1/3 upper), descending colon / colon descendens (1/3 distal) and sigmoidal colon / colon sigmoideum (Table no. 1).

Table 1. Meconium volume and distribution into the intestinal sectors

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A. Macro anatomic analysis of the meconium location in fetus on gestation age

We identified a ball of intestinal ansae without sectorial differentiations and meconial content, in the upper half of the peritoneal cavity, in a 13 week fetus (10 cm vertex-coccygis length). Large intestine primordial had a sinusoidal descending traject connected to the anal channel (Fig. 1 A-C).
Fig. 1 Meconium volume and stereodistribution variability within the intestine and colon sectors in 13 week (A-C), 18 week (D-F), 19 week (G-J) and 20 week (J-L) foetuses.
Fig. 2 Meconium transit and its accumulation in rectum and distal colon in 22 week (A-C), 24 week (D-F), 26 week (G-I) and 28 week (J-L) foetuses.
Fig. 3  A-F. 20 week foetus (19 cm) with meconial plug on the ileum sector of the small intestine, with effects on the meconium transit to the colon sectors. Intestinal lumen is occupied by the hard meconium, on sections, sagitally.
G-I. Congenital umbilical hernia with externalized liver. Intestines enlarged by a hard meconial content.
Fig. 4 Phenotypical changes of the ileum structures according to the meconium variable quantity accumulation. *H-E* staining. Oc. 7, Ob. 10 x (A, E large image); 20 x (C); 40 x (B, D, E)
We observed the presence of a meconial plug into the ileal intestinal ansae, distal colon spasticity, meconium accumulation into both the rectum and sigmoid colon and the absence of meconium both into the proximal colon and intestinal jejunum ansae in an 18 week fetus (17 cm vertex-coccygis length). Meconium was present in a 19 week fetus (18 cm vertex-coccygis length); it had a homogenous distribution and it was fluid in all the sectors of both the small and large intestines. We identified haustra on the distal colon and a dextropositionning of the sigmoid colon which became an omega-shaped one (Fig. 1, D-F).

**In the 20 week fetus** (19 cm vertex-coccygis length) the intestinal ansae from the ileal sector had a widening traject and they contained hard meconium. On sagittal paramedial sections, the lumen of those ansae were blocked with meconium plugs (Fig. 1, J-L). We easily identified a huge accumulation of viscous meconium into the proximal colon sectors excepting caecum, into the distal colon sectors and first of all in rectum, in a **22 week fetus** (22 cm vertex-coccygis length) (Fig. 1, G-I).

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**In a 24 week fetus** (25 cm vertex-coccygis length) the sectors of the distal colon were enlarged by a quasi-solid meconial content determining changes in the form and the traject of the descending and sigmoid colons. Small quantities of meconium were present into the proximal colon sectors (Fig. 2, D-F).

**In a 26 weeks fetus** (27 cm vertex-coccygis length) it could be easily observed the filling of fluid meconium of all the proximal and distal colon sectors. Ileo-cecal junction area was spastic, without meconial content above that area, meconium was present in small quantities at the ileum and jejunum sectors of the small intestine (Fig. 2, G-I).

**In a 28 weeks fetus** (30 cm vertex-coccygis length) large accounts of meconium occupied the proximal colon sectors, excepting caecum and distal colon increasing the spleen flexure and the appearance of a flexure, frontally in the 1/3 lower descending colon. Many haustra crossed all the colon sectors. Ileoceleal junction was lack of meconium; it was white-grayish and interfaced between the proximal and distal sectors full of meconial liquid (Fig. 2, J-L).

Two cases drew our attention especially by the forming of the "meconial plug" on sectors of a variable extent. In a case of a plurimalformed fetus, with a hepato-intestinal eventration, the meconial plug had been constituted in each sectors of both the small and large intestines (Fig. 3, G-I). In the second case, the meconial plug was present into three ileal ansae, enormously enlarged, having a winding traject (Fig. 3, A-D). Small amounts of meconium were present into jejunum and absent in all the sectors of both the proximal and distal colons (Fig. 3, B-D). On sagittal sections performed through the sector containing the "meconial plug" the ileal ansae lumen was occupied by the hard meconium (Fig. 3, F).

**B. Micro anatomic analysis of the phenotypical changes of the intestinal wall structures in the meconium accumulations**

The presence of structural changes at the level of both the mucous membrane and muscular layer could be observed from the analysis of seriated sections performed through the ileal sector of the small intestine, under the condition of variable meconium accumulations. In the case of minimum accumulation of meconium, intestinal villus were surrounded by small meconial conglomerates of a filamentous appearance adhering to the villositary epithelial surface (Fig. 4, A). By using a 20x examination objective, we easily observed meconium penetration into the intervillositary spaces to which it contracted contiguity relationships (Fig. 4, B). In the cases of mean quantity of meconium accumulations, a process of densification and forming of pseudopodia extended to the intestinal villus took place into the intestinal lumen.
Similarly, we could observe a reduction of the intestinal villus heights and an intestinal crypts flattening tendency (crypta intestinalis = glandule intestinalis) (Fig. 4, C).

By using a 40x objective we observed that intestinal villus appeared adherent to the villositary epithelium. At the epithelio-mecional junction place, apoptosis appeared, hypertrophy of enterocytes containing nuclei placed at the basal apex and, here and there they were in division and a hyperactivity of intestinal stem cells present in the intestinal crypts area, as well (Fig. 5, A, B). By examining with 20x and 40x objectives, a part of the intestinal villus bare remolding processes and phenotypic changes in elipsoidal structures (Fig. 5, C, D).

In the case of huge hard meconium into the terminal ileum, we observed the general atrophy of the mucosa by the centrifugical compression produced by the meconial plug (Fig. 4 E). By examination with 20x objective the terminal ileum all, we noted the meconial plug adherence to the mucosa, partly transformed by changing processes. Muscular layer was dissociated by a strong interstitial edema (Fig. 4 E).

In the conjunctivo-vascular axis of the intestinal villus conterminous to meconium accumulations, we identified nodular lymphocyte infiltrates (Fig. 6, A-C). Muscular layer of the terminal ileum wall were dissociated by a strong intestinal edema. We identified micro-neuroganglia of the mienteric plexus into the interstitial between the intestinal and external muscular layer (Fig. 6, D, E).

DISCUSSIONS
Meconium is an exceptional particular phenomenon in the human biology knowledge during the antenatal stage of ontogenesis. It was formed starting the forth month of ante partum life by the contribution of three fluids such as: gastrointestinal juice, billa and the amniotic liquid. Meconium composition and consistence, during transiting the digestive tract, were variable and dependent on absorption potentialities of the intestinal mucosa and on the content coordinated propulsion by the intestinal peristalsis. Within the period of 4-9 months, epithelial desquamated cells form the skin and intestinal mucosa, vernix caseosa and lanugo, all of them remained stored into the intestine [11, 16].

By analyzing our observations, we noted the following: progressive increase of the meconium viscosity to the „distal colon” sectors and its accumulation starting with the rectal sector; volume enlargement of the proximal and distal colon sectors following the meconium storing, determining the hepatic and spleen flexures, increase and the new ones appearences into the sigmoid jonction; haustra is determined by the contraction of the fascicles of circular muscle fibers of the colon muscle tunic; ileocecal spasticity; the presence of „meconial plugs” into the ileum sector of the proximal colon causing the meconium transition through the intestine to stop (meconial ileus); phenotypic changes of the vilosities and intestinal crypts at their contact to the meconial store.

Knowing the signification of the meconium elimination moments is a must for different specialists such as in obstetrics, neonatology and legal-medicine [8, 11, 15, 16].

In an alive new born, meconium is present in all the colon sectors. It is considered that normally, meconium elimination takes place during the first 2-3 days postnatal. A fetus dead during its birth has its meconium into the distal colon. Meconium excretion during its birth represents a pathologic process. Meconium can be eliminated ante partum, too, into the amniotic fluid because of the neural centers excitation by hypercapnia. In the prenatal dead fetuses meconium is mixed with blood and amniotic fluid.

Meconium transit can be delayed or blocked in many diseases: Hischprung’s disease, anorectal malformation, left small colon syndrome, hipoperistaltism syndrome in mega cystic micro colon, meconial plug syndrome, meconial ileus, hipoganglioniosis. Meconium biochemical content is toxic by fat acids especially the oleic acid on the epithelial membrane [3].
Fig. 5 Intestinal villus and crypts. 1. Epitelium simplex columnare; 2. Vas lymphaticum centrale; 3. Cripta intestinalis; 4. Location of the intestinal stem cells; 5. Exocrinocytus calciformis. H-E staining. Oc. 7, Ob. 20 x (A, C); 40 x (B, D).
Fig. 6 Lymphocitary infiltrates in lamina propria villi (B, C). Stasis and edema into the muscle layer. „Entheric neurons” of the mienteric nervous lexus were visible between the circular and longitudinal layers. 1. Meconium. 2. Villus intestinalis. 3. Lamina propria villi with lymphocytary infiltrates. 4. Entheric neurons.
The presence of the lymphocyte infiltrates into „lamina propria villi” (Fig. 6 B), of the villosities form the proximity of the meconial accumulation represents an often phenomenon. It was observed in vitro, that neutrophiles exposed to meconium released IL-8 that increased the inflammatory response by neutrophyles multiplication.

A special question is raised by meconium advancing through the intestin. Two phenomena were visualized when we macro anatomically analyzed our cases: the established meconium absence into the ileocaecal sector (Fig. 1 K) and the existence of the ring-shaped, sector contractions both at the level of the „distal colon” (Fig. 1C) and the „proximal colon”. Meconium transit is regulated by various hormones such as motilin, a 22 aminacids polypeptide; it induced the propulsion movement of the meconial ball.

The presence of a sphincterian system at the ileo-caecal jonction level can explain the permanent vacuum state of that sector.

Meconium, by cytokines and other associated factors is an implemented pathomorphogenesis processes. It can induce vasoconstriction determining tissue reduce irrigation, including the cerebral one [Altshulep, 1989] but also the necrosis of the blood vessels from the umbilical cord [23].

Carl von Rokitansky (1804-1878) described the death of a fetus with meconial peritonitis, a complication of the meconial ileus [2].

Landsteiner (1905) is the first who observed the association between the cystic fiborsis and the meconial ileus [13].

Drug abuse during pregnancy is a very important public health problem [10, 12, 20].

Meconium study brings reliable elements for fetus exposure to drugs used by the mother during her pregnancy. It is considered that the fetus eliminates drugs into the bile on the hepatic way but also placentl into the amniotic fluid: they were then accumulated in meconium either directly form bile or indirectly, by swallowing the amniotic fluid.

By means of the chromatographic and immunochemical methods, a wide range of drugs can be determined: cocaine, amphetamines, opiates, canabinoids or phencyclidines [1, 5, 7, 17].

As to prevent the possible losses of drugs, meconium sample must be sent to the laboratory as soon as it was removed. If the sample is kept for 24 hours at room temperature, a decrease of the cocaine and canabinoid rates can occur. Including meconium into a solvent (buffered methanol) can prevent the decrease of the drug concentration rate for 72 hours. For prolonged storing, meconium should be frozen at -15ºC [1, 14, 21].

CONCLUSIONS
1. Meconium accumulation, location, composition and consistence into the intestine have been variable in time and space and they can represent reliable elements in evaluating the relationships among gestational age, meconium intestinal transit, prematurity, birth and / or death.
2. Meconium should be used to detect drugs into the foetus who was exposed to them by the pregnant woman who used them.
3. Ileo caecal sphincter barrier is the determining factor of the first meconium accumulations in the ileum region of the small intestine; it represents the key element in the meconium plug formation and in the pathophysiology of meconial peritonitis.
4. The distal colon, by regional contractions assures the meconium transit to the rectum, as the first collecting station.
5. The processes of reciprocal induction between the functional structures of the intestine and meconium ensure the anatomic stability of the sub diaphragmatic alimentary tract.
6. Meconium induces phenotype transformations inside intestinal villosities and crypts; it determines the appearance of the lymph infiltrates in lamina propria vili and provokes a hyper activity of the Stem cells inside intestinal crypts.
7. Fetus exposure to illicit drugs utilized by the mother can be proved by chromatographic or immune hystochemical analysis of meconium.
References