Histopathological Placental Screening as Valuable and Non-Invasive Method for Assessing Etiology of Second Trimester Recurrent Abortion

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Abstract: This study is an evaluation based on placental screening, of the importance of ascending intrauterine infection and placental vascular pathology. The paper is as well a plea for the routine use of the modern histopathological exam of the products of conception along with the noninvasive screening of chorioamnionitis (by means of serum C-reactive protein measurement) in the evaluation of late abortion (the recurrent one in particular) since the ascending intrauterine infection and placental vasculopathy seem to play an important part in the pathogenesis of this type of miscarriage. The therapeutic measures against this particular type of preterm labor should therefore focus on either bacterial vaginosis (the world most frequent cause of recurrent abortion of infectious origin) or/thrombophilia even before the standard lab results are available.

Key-Words: Placental screening, Ascending intrauterine infection, Recurrent abortion, Vascular pathology of placenta, Late miscarriage

1 Introduction
Romero and his coworkers [1,2] and Arias with his team [3] have filled in, and Rai et al’s studies [4,5,6,7] and Spong et al’s studies [8] have supported the valuable notion of the placental histopathology screening for the ascending intrauterine infection injury and for the placental vascular injury.

Many morphopathology observations of the concept product from the studies of Rai group’s [5,6] are systematically detecting, but in various proportions, in the late miscarriage and even in the preterm births (that are overlapping, between 20-28 gestational weeks [10,11] ), the placental ischemia and/or the acute decidual chorioamnionitis pain, like the most often involved pathological processes in the multifactor etiology of the preterm labor, slightly understood [12,13,14,15,16].

Arias and his coworkers [3], according to Spong and his coworkers [8] are mentioning that 70% of preterm labors, with or without premature rupture of membranes, are the result of ascending intrauterine infection and placental vascular pathology. And if very large studies will confirm these observations, that means that desperate efforts to inhibit premature uterine activity, well installed, in order to prolong the pregnancy, are unnecessary not only because of the lack of efficiency (according to Rust et al [17] that shows that in advanced labor the conventional tricilysis is inefficient), but also because the extended pregnancy keeps the fetus in a hostile environment, that more is harming than supporting.

Like Arias group [3] , Rai and his coworkers [6,7], and also Dizon-Tawson and his coworkers [18] are suggesting that the histopathological examination of placenta, which reveals a placental vascular pathology like placental infarction expanded to over 10% of the surface, with or without calcifications (Rand et al [19]) and/or accelerated streaking of the placenta villi next to the multiple syncytial nodules or chorioangiosis placenta , possible the absence of adaptation of the spiralate decidua arterioles is a practical screening in recurrent undue abortions, preterm labor and the delay growth or fetal death in the uterus.

Is not clear the nature of the link between the absence of an appropriate hemochorial placentation and such a varied clinical expression, from the pre-eclampsia to the delay growth or fetal death in uterus, or preterm labor or premature rupture of membranes and recurrent abortion in the second trimester of pregnancy.

The groups Arias  [3], Rai [6,7] and Dizon-Tawson [18] in agree with the observation of the
arterial flux utero and fetal-placental trough ultrasonography [20], agree with the possibility that the deficiency of the trophoblast could generate changes in the spirulate arterioles, which is the cause of unequal and improper utero-placental blood flow which will be the basis of accelerated maturation, with numerous syncytial nodules and fibrosis of the placental villi and at the same time of the placental attacks, by diverse intensity, knowing, on the other hand, that the thrombophilic issues could generate extended placental thrombosis, or placenta defects and embryonic implantation issues.

The thrombophilia can be wined (primary and secondary antiphospholipid syndromes and nefrotical syndrome) or congenital - the quantitative and qualitative constitutional deficits in the natural inhibitors of the coagulation, meaning C-protein and S-protein and antithrombin, or mutations of the target factors of this inhibitors, mutations that are inhibiting their actions (Leiden mutation of the V factor and that of the prothrombin or the G202A10 mutation) [21].

According to Burns group [22], in the relation infection-recurrent abortion must be establish if there is a connection between the genetic predisposition for the infection and the recurrent abortion or if it is possible to delegate a strong association between specific groups of vaginal bacteriosis and the pregnancy prognosis [1,3,8].

The causative role of the preterm recurrent labor of the vaginal infections is a new field of research [22] that seems to orientate with predilection on the vaginal bacteriosis, defined as a alteration of the vaginal flora, where the number of the lactobacillus which usually predominate, is very low or they are absent [24,25,26,27].

Our retrospective clinical-morphopathological study of the miscarriage in “Filantropia” Maternity from Craiova, was made in light of modern anathomopathological screening of the concept product in an attempt to find explanations for the emergence of extremely premature labor and the extremely premature rupture of membranes (in the second trimester of pregnancy) and eventually in finding out solution to prevent them by comparing the results of clinical-morphopathological exam with the evaluation of women who have a normal pregnancy and gave birth at term.

2 Material and Methods
We have studied 59 placentas from late miscarriages between 14 -27 gestational weeks in the “Filantropia” Maternity from Craiova, excluding multiple pregnancy and fetal malformations. The 59 placentas were analyzed retrospectively clinical-anatomopathological.

We have used for control the clinical-morphopathological data from 40 normal pregnant women who gave birth at term, because the majority of miscarriages studied occurred between 20-27 weeks of gestation, so they can be framed in the category of extremely preterm births [10,13].

Clinical information was obtained from case report. It includes demographic data, gestational age, obstetrical history, health status at admission and laboratory investigations. In the studied group of 59 late miscarriages were recorded as associated diseases: recurrent abortion in 56 cases and premature rupture of membranes, in less then 24 hours, in 35 cases.

The morphopathological examination of the placentas was performed by the pathologist without knowing the clinical details of the case following the Gaillard [28] protocol, which can be summarized: placenta fixed in buffered formalin 10% was weighed, measured and then sectioned for taking fragments from all its thickness, from the extraplacentary membranes and from the umbilical cord to put it in paraffin .That preceded the microscopic study of the sections stained with hematoxylin-eosine or Van Gieson from the fragments above mentioned.

Histological,chorioamniotitis was characterized by polymorphonuclear leucocytes, with or without necrosis present in the fetal membranes and subcorial fibrin [3].

Fig.1 Acute chorioamniotis. Polipoid aspect of amniotic membrane with rich inflammatory exudates in the chorioamniotic axe; edema and exudates in the capsular decidua- the severity of the amniocoriodecidual granulocyte reaction suggests just like the acute marginal choriiodecidual inflammation the ascending intrauterine infection (Hematoxylin-eosina; ob.10)

The severity of the polymorphonuclear infiltrate (Fig. 1,2,3) was based on the number and the degree of infiltration of neutrophils, their marginal and mixed choriodecidual disposal, vascular umbilical
disposal and in chorial board. Was also based on the presence of the limphoplasmocitary infiltrate with or without vascular degeneration of the fetal membranes, sometimes even with morphopathological signs suggesting placental insufficiency [1,3,12,29].

Fig. 2 Umbilical vasculitis. Acute intravascular and intraparietal inflammatory exudates, parietal edema. Umbilical vasculitis, as funiculitis, is an indicator of maximal specificity (higher positive predictive value) of microbial invasion of amniotic cavity (Hematoxylin-eosina; ob.20)

The sever maternal and fetal placental vascular pathology was diagnosed [18,19] by the presence of many placental infarctions (exceeding 10% of placental surface) in addition with either umbilical vein thrombosis (Fig.4) or suggestive calcification of aggression of the antiphospholipid antibodies type IgM (Fig.5) and the uneven accelerated maturation of chorionic villi, sometimes associated with multiple multinucleated syncytial nodules (Fig.6).

A mixed lesion consisted in a coexistence of taint with maternal and/or fetal placental vascular pathology [3].

Chronic villitis / hemorrhagic endovascularitis were characterized by chronic inflammation areas with mononuclear cell infiltration, while fibrinoid necrosis areas affected groups of vilosities and the granulocytes were missing. They were also characterized by hematic infiltration with vascular dystrophy [12].

Abruptio placentae was diagnosed when a retroplacental thromb left a final impression on the
maternal surface of the placenta, impression that is histopathological obvious [3].

A value of \( p<0.05 \) had a statistical significance at the comparing the results of the Mann Whitney U test and \( \chi^2 \) (chi square) test – with the Fisher correction for the small groups [30].

### 3 Results and Discussions

The descriptive variables (demographics) for the patients of the three groups (late miscarriage with intact membranes, late miscarriage with premature rupture of membranes and the control group), clinical-morphopathological evaluated, are presented in Table 1.

#### Table 1 The demography of the patients with late miscarriages versus group control

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Abortion with intact membranes No.=24 (average +/- standard error of the average)</th>
<th>Significance (Mann Whitney U test)</th>
<th>Control group No.=40 (average +/- standard error of the average)</th>
<th>Significance (Mann Whitney U test)</th>
<th>Abortion with premature rupture of membranes No.=35 (average +/- standard error of the average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23 +/- 0.82 Insignificant</td>
<td>25.6 +/- 4.3 Insignificant</td>
<td>25 +/- 2.4 Insignificant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>0.12 +/- 0.1 Insignificant</td>
<td>0.23 +/- 0.15 Insignificant</td>
<td>0.1 +/- 0.1 Insignificant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>23.2 +/- 2.1 P&lt; 0.05</td>
<td>39.02 +/- 1.2 P&lt; 0.05</td>
<td>24.1 +/- 2.3 P&lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2 The classification of the patients with late miscarriages made according to the clinical-morphopathological results

<table>
<thead>
<tr>
<th>Results</th>
<th>Miscarriage with intact membranes No.= 24</th>
<th>Significance (chi square test)</th>
<th>Control group No.=40</th>
<th>Significance (chi square test)</th>
<th>Miscarriage with premature rupture of membranes No.=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending intrauterine infection</td>
<td>5 (20,8%)</td>
<td>P&lt;0,05</td>
<td>4 (10%)</td>
<td>P&lt;0,05</td>
<td>9 (25,7%)</td>
</tr>
<tr>
<td>Placental vascular pathology</td>
<td>16 (66,6%)</td>
<td>P&lt;0,05</td>
<td>5 (12,5%)</td>
<td>P&lt;0,05</td>
<td>18 (59,3%)</td>
</tr>
<tr>
<td>Mixed infectious and vascular lesions</td>
<td>1 (4,1%)</td>
<td>Insignificant</td>
<td>2 (5%)</td>
<td>Insignificant</td>
<td>3,8 (8,5%)</td>
</tr>
<tr>
<td>Chronic villitis/ Hemorrhagic endovasculitis</td>
<td>0 (0%)</td>
<td>Insignificant</td>
<td>1 (2,5%)</td>
<td>Insignificant</td>
<td>1 (2,8%)</td>
</tr>
<tr>
<td>Abruptio placentae</td>
<td>1 (4,1%)</td>
<td>P&lt;0,05</td>
<td>0 (0%)</td>
<td>Insignificant</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Without previous placenta lesions</td>
<td>1 (4,1%)</td>
<td>P&lt;0,05</td>
<td>28 (70%)</td>
<td>P&lt;0,05</td>
<td>4 (11,4%)</td>
</tr>
</tbody>
</table>
As expected, we can notice a significant difference (Mann Whitney U test, p<0.05) between control group and the groups we have studied (late miscarriage with and without premature rupture of membranes) only in terms of gestational age at which the conception product was expelled. That means that for the three groups there are equal risks of infection and placental vascular pathology.

Table 2 presents the classification of patients in the following categories: infection (ascending intrauterine infection), placental vascular pathology, mixed lesions, chronic villitis/hemorrhagic endovasculitis, abruptio placentae and normal aspect (without previous placental lesions), according to the definitions set in subsection “Materials and methods” and partially exemplified in Fig 1-6.

Table 2 shows that:

a) The prevalence of intrauterine infection and placental vascular pathology was significantly higher at patient with late miscarriage with intact membranes and premature rupture of membranes compared to control group who gave birth at term.

b) Abruptio placentae is significantly more common at the patients with late miscarriage compared to control group.

c) The number of patients with normal aspect of the placenta (without the lesions in Table 2) was higher in the control group compared to the group with late miscarriage with intact membranes and premature rupture of membranes.

d) Chronic villitis and hemorrhagic endovasculitis were not so common in the control group and in the groups we have studied.

e) Surprising was the observation of the prevalence of ascending intrauterine infection, which was significantly undefined between the group of patients with late miscarriage and intact membranes and the group of patients with late miscarriage and premature rupture of membranes. This observation is supported by similar results of the Salafia group [12] and indirectly by Gaillard and his coworkers [28]. They find out that more than half of the 420 patients who had miscarriage in the second trimester of pregnancy with intact membranes had the amnioculture positive.

f) Also, the two groups studied with late miscarriage and premature rupture of membranes or intact membranes are not significantly different in terms of incidence of placental vascular pathology which allows a joint evaluation of the two studied groups. The evaluation indicates on the population with late miscarriage with premature rupture of the membranes or intact membranes a 57.6% prevalence of placental vascular pathology, and a 23.7% prevalence of ascending intrauterine infection. That means that the etiopathology of late miscarriage and in other words of very preterm birth (only 11.2% of patients with late miscarriage were under 20 gestational weeks) from “Filantropia” Maternity from Craiova is dominate by the placental vascular pathology and ascending intrauterine infection in 81.3% of cases. This value is close to 71% found by the Arias group at the St. John’s Mercy Medical Center, St. Louis [3], by adding the placental vascular pathology cases (with incidence around of 34% regardless of status membranes) with those affected by ascending intrauterine infection (with incidence of 37% regardless of membranes status). They have been verified on basis of standard criteria clinical-anathomopathological (similar to those used in this evaluation) and microbiologically (cultures of placental tissues) examined among the 105 cases of preterm birth.

The domination of the etiopathology of late miscarriage and preterm birth by the placental vascular pathology and the ascending intrauterine infection noted here is in agreement with the results of other international groups [8,12,31]. Study findings, among the 59 late miscarriage with or without premature rupture of membranes, of a 23% prevalence of ascending intrauterine infection is similar to that identified by assays of C-reactive protein in a group of 58 patients with miscarriage in the second trimester of pregnancy who were interned in obstetrics-gynecology clinics of the University of Medicine and Pharmacy from Craiova [32]. This argues in favor of the current study, apparently limited number and selective (59% premature rupture of membranes, >90% recurrent miscarriages), and on the other hand recommends the dosage of CRP in the second trimester of pregnancy as a non-invasive screening, fast and not expensive, of high urogenital infection which allow the tempest application of modern therapy in a preterm labor of infectious origins.

The research of Quin’s group [33] are revealing the fact that the human infection with Ureaplasma urealytium is capable to induce the presence of antiphospholipid antibodies which are responsible for thrombotic placental lesions [19].

There were found histopathological lesions with Ureaplasma urealytium [29] in the sections of 4 cases with vascular pathology of the placenta, cases that can be incorporated in the population with ascending intrauterine infection.

The incorporation of the four cases of placental vascular lesions and all mixed lesions from our statistic in the ascending intrauterine infection is
correcting the 23.7% and 57.6% prevalence, allowing in this way an almost perfect overlap between the incidence of the ascending intrauterine infection and vascular pathology of the placenta on the population with late miscarriage (very preterm birth) investigated here with the one observed by Arias group [3] on a group almost double of preterm births (including the very preterm births).

This overlap is a confirmation of the power of this study and the validity of its results.

Because of over 42% of extended placental thrombosis are caused by the Leiden mutation of the V factor [18], the most frequent genetic predisposition to vein thrombosis and because the placental vascular pathology has affected almost 50% of cases we can say that this study is an argument for introducing the placental screening in the investigation of the ethiopathology of late miscarriage. It is also an argument for adopting an early exploration of the C-reactive protein resistance which once detected in a patient with abortion, especially iterative one, will impose the exploring of her relatives, which will increase efficiency, not only of iterative abortion, disprove by starting the antithrombotic therapy before conception [6,7], but the risk of thrombosis in general population.

If another study of the same type presented here, but larger, of miscarriage, especially iterative one, will confirm at the population of “Filantropia” Maternity from Craiova, the dominance of ascending intrauterine infection and the placental vascular pathology in the ethiopathology of preterm labor, in the condition of excluding the possibility of placental vascular pathology in the asymptomatic phase of the ascending intrauterine infection by the CRP serum screening. [32] will appear the possibility of increasing the efficiency of the first prophylactic and curative measures in the extremely preterm labor before obtaining the histopathological examination results from the previous miscarriage.

This evaluation draws the attention to the large presence of subclinical chorioamnionitis, because none of the cases with positive histology of chronic chorioamnionitis (according to Romero group [1,2]) investigated here, had no fever, maternal tachycardia or leucocytosis. The high frequency of subclinical chorioamnionitis is not something new [1,2,3,12]. They appear as dominant (over 80% of the cases of late miscarriage) in the ethiopathology of late miscarriage (preterm births). The study draws the attention to the necessity of the routine application of the modern histopathological examination in the investigation of the miscarriage in the second trimester of pregnancy. It also reiterates the importance of another non-invasive screening - CRP serum screening. So the first therapeutic and curative measures of extremely preterm labor can be orientate to combat vaginal bacteriosis, trombophilia with the CRP-serum level normal or higher.

The study draws the attention also to the application of modern evaluation of iterative abortion. Because the placental vascular pathology is more common than the infection is necessary to introduce the exploration of the C-reactive protein resistance (the most frequent genetic predisposition of thrombosis).

4 Conclusion

This study is an evaluation based on placental screening, of the importance of ascending intrauterine infection and placental vascular pathology (the thrombophilya has a central place). The study draw the attention also to the necessity of the routine application of the modern histopathological examination in the investigation of the miscarriage in the second trimester of pregnancy. It also reiterates the importance of another non-invasive screening - CRP serum screening. So the first therapeutic and curative measures of extremely preterm labor can be orientate to combat vaginal bacteriosis, trombophilia with the CRP-serum level normal or higher.

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