REVIEW

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Histological, immunohistochemical and clinical considerations on amniotic membrane transplant for ocular surface reconstruction

ELENA ŞAPTE[1)], CLAUDIA FLORIDA COSTEA[2)], ALEXANDRU CĂRĂULEANU[3)], CRISTINA DANCĂ[4)], GABRIELA FLORENŢA DUMITRESCU[5)], GABRIELA DIMITRIU[4)], MĂDĂLINA ADRIANA CHIHAIA[4)], CĂTĂLIN MIHAI BUZDUGĂ[6)], ANDREI CUCU[7)], MIHAELA DANA TURLIUC[7,8)]

[1)]*Department of Anatomy, Faculty of Dental Medicine, "Ovidius" University of Constanţa, Romania*
[2)]*Department of Ophthalmology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[3)]*Department of Obstetrics and Gynecology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[4)]*2nd Ophthalmology Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[5)]*Laboratory of Pathology, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[6)]*Department of Endocrinology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*
[7)]*2nd Neurosurgery Clinic, "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iaşi, Romania*
[8)]*Department of Neurosurgery, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

Abstract

Amniotic membrane (AM) transplantation has been used successfully worldwide in ophthalmology plastic surgery for over 100 years. This review presents the histological and the immunohistochemical features of AM compared to those of the conjunctiva and discusses the techniques of processing and preservation, its mechanism of action in ocular reconstruction, its clinical ophthalmic indications, but also advantages and limitations of grafting with this biomaterial.

Keywords: amniotic membrane, transplant, conjunctiva, limbal stem cells, cytokeratins.

Introduction

The amniotic membrane (AM) is the inner vascular layer of the three-layered fetal membrane [1]. It is located on the inner layer of placenta and fully surrounds the embryo, outlining the amniotic cavity, which is filled with amniotic liquid [2].

The first therapeutic use of AM was successfully achieved in 1910, by the American surgeon JW Davis, who used it in skin grafting in Johns Hopkins Hospital [3]. The first ocular therapeutic indication of AM was suggested by de Rötth, in 1940, for treating a chemical burn of the ocular surface [4].

Sorsby & Symons (1946) [5] and Sorsby *et al.* (1947) [6] used "dry" amniotic membrane, termed "amnioplastin", as a temporary patch for treating acute ocular burns.

In 1995, Kim & Tseng reintroduced the use of amniotic membrane for ocular reconstruction. They showed on a rabbit model that 40% of the corneas with total limbal deficiencies may be reconstructed by replacing the conjunctival surface with the preserved amniotic membrane [7].

The amniotic membrane had been used up to that time in ophthalmology as a graft for corneal and conjunctival reconstruction in a variety of ocular surface diseases, as a biological patch or bandage, to treat acute inflammatory disorders, and as a carrier of limbal stem cells [8].

Comparative histology: conjunctiva *versus* amniotic membrane

The conjunctiva is a thin transparent mucous membrane, which protects the eye from infection, lines the inside of eyelids, and surrounds the entire globe, except for the cornea. From the microscopic point of view, the conjunctiva is made up of two layers: the conjunctival epithelium and the subepithelial stroma (Figure 1). Under the mucosal surface of the conjunctiva, there are numerous patches of lymphoid tissue and a rich supply of blood vessels and lymphatics. At the limbus, the conjunctival epithelium becomes the corneal epithelium [9, 10].

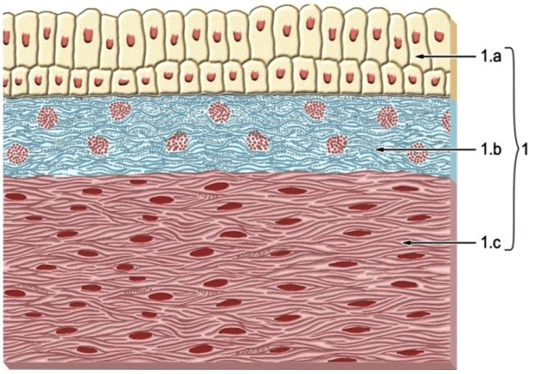

Figure 1 – ***Histology of the conjunctiva: 1.a: Epithelium; 1.b: Adenoid layer; 1.c: Fibrous layer (after Sava et al. [10]).***

The conjunctival stroma is a highly vascularized connective tissue separated from the epithelium by an underlying membrane [10]. It includes two sections: (*a*) the adenoid surface layer, which is not present at birth, but emerges after the age of three months, is made up of reticular connective tissue including lymphocytes that in some areas comprises follicle-like structures with no germinal centers (Figure 2); (*b*) the fibrous deep layer consisted in connective tissue with collagen and elastic fibers and is, thicker than the adenoid layer, with the exception of the tarsal conjunctiva. The latter adheres to the tarsal plate and belongs rather to the subconjunctival tissue than to the conjunctiva. The fibrous layer stretches loosely over the eyeball. Nerves and glands are found in the conjunctival stroma [10].

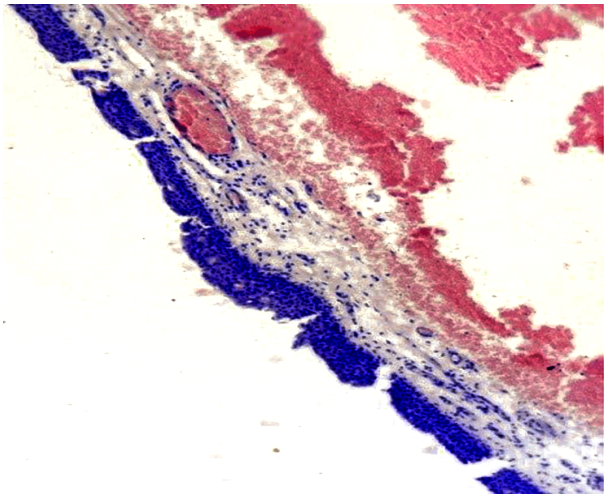

Figure 2 – ***Microscopic aspect of bulbar conjunctiva: stratified squamous epithelium situated on the stromal adenoid layer [Hematoxylin–Eosin (HE) staining, ×100] (after Sava et al. [10]).***

The basement membrane of the AM closely resembles that of the conjunctiva and cornea especially with regards to its collagen composition [11]. There are similarities between the conjunctival graft and the amniotic membrane, which is why both of them can be used to support the weak cornea. Being a vascularized tissue, the conjunctival flap provides fibroblast cells and white cells in the cornea, supporting the healing of corneal ulcers. Conjunctival flaps may be used to cover the cornea in pterygium and conjunctival neoplasm excision and in the reconstruction of the injured cornea or corneal scar [9].

AM is made up of extra-embryonic tissue and consists of a fetal component (chorionic plate) and a maternal component (the deciduas) (Figure 3). These two parts are held together by chorionic villi and they are connected by the cytotrophoblastic shell of the chorionic sac to the *decidua basalis*. The fetal component separates the fetus from the endometrium. The amnio-chorionic membrane outlines the external borders of the sac, which encloses the fetus, whereas the innermost layer of the sac is the amniotic membrane [12].

Histologically, AM is represented by a single layer of metabolically very active cuboidal to columnar epithelium with microvili (Figure 4), firmly attached to a basement membrane, and an avascular and relatively sparsely populated stroma [11, 12]. The basement membrane is made up of type IV, V and VII collagen (also found in conjunctival and corneal basement membranes) in addition to fibronectin and laminin [11, 13]. It is one of the thickest membranes in the human body and can withstand current cryopreservation techniques. The stroma is further divided into three contiguous, but distinct layers: the inner compact layer, which is in contact with the basement membrane and contributes to the tensile strength of the membrane, middle fibroblast layer, which is thick and made up of a loose fibroblast network, and the outermost spongy layer [11].

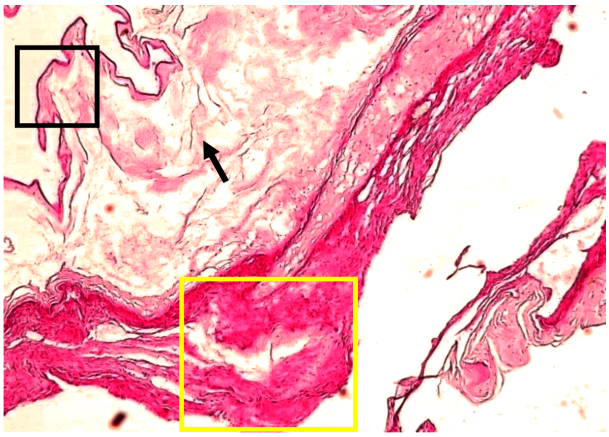

Figure 3 – ***The human amniotic membrane lines the fetal environment during gestation, separating the developing fetus from his/her mother in utero. Black square: Amniotic membrane or fetal side showing a single layer of cuboidal epithelial cells, basement membrane and stromal layer. Yellow square: Maternal deciduas made up of trophoblasts situated on a basement membrane, and a stromal layer. Black arrow: Between amniotic membrane and chorionic layer, there is the intermediate layer made up of mesechyme showing fibroblasts (HE staining, ×100) (private collection, Dr Gabriela Florenţa Dumitrescu).***

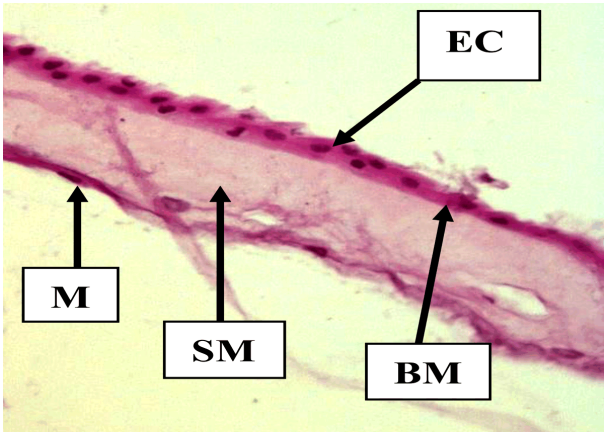

Figure 4 – ***Magnified view of the black rectangle frame from the previous figure, showing the microscopic structure of amniotic membrane (HE staining, ×400) (private collection, Dr Gabriela Florenţa Dumitrescu). EC: A single layer of cuboidal epithelial cell with microvilli; BM: Thick basement membrane; SM: Avascular stromal matrix; M: Mesenchymal intermediate layer made up of fibroblasts.***

There are no nerves, muscles or lymphatic vessels in AM. Amnion nutrition is achieved by oxygenation from the chorionic surrounding fluid, amniotic fluid and fetal surface vessels [14].

Comparative immunohistochemical phenotypes: conjunctiva *versus* amniotic membrane

Simple AM transplantation requires cells of surrounding healthy conjunctiva to move out and cover the surface of the amniotic membrane in order to achieve re-construction of the eye surface [15]. For serious defects of the conjunctiva, simple AM transplantation is challenging. It has been reported that successfully transplanted human AM can transform into conjunctival epithelia-like cells and that the surrounding conjunctival tissue is sufficient to create the microenvironment required for directional differentiation of amniotic epithelia [16–19].

AM can be obtained from donors undergoing elective cesarean section, and who have been previously screened serologically for potentially communicable diseases including human immunodeficiency virus, hepatitis B and C viruses and syphilis [7, 20].

AM has similar histological components as the basal membrane of ocular superficial epithelium and can secret cytokines such as alkaline fibroblastic growth factor and epidermal growth factor to promote epithelial adhesion, migration, and induced differentiation [19, 21–24]. The immunohistochemical staining shows that the epithelium of both types of tissues expresses common cytokeratins (CK) AE1/AE3 and CK 19 and this is the proof that the amniotic membrane could be used with success in conjunctival repair of the ocular surface (Figures 5–8).

On the other hand, amniotic membrane stroma is endowed with growth factors, among which the transforming growth factor-β (TGF-β) and epidermal growth factor are vital. The exact mechanism of its action has not been clearly defined yet, but in most instances, it is widely accepted that it acts as a substrate, which is very conducive to epithelial cell migration and attachment [11].

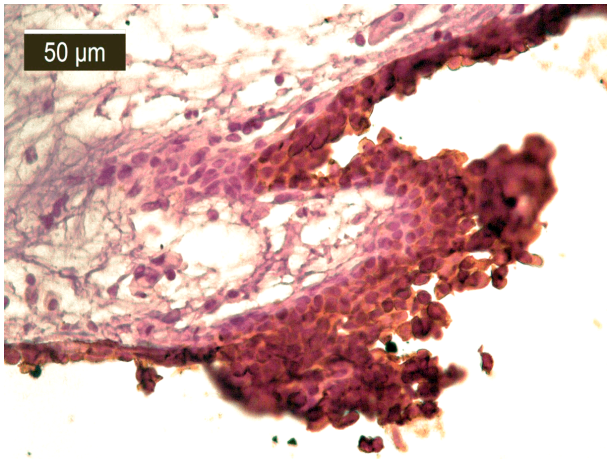

Figure 5 – ***Conjunctiva: epithelial cells shows immunopositivity for CK AE1/AE3 (Immunostaining, anti-CK AE1/AE3 antibody, ×400) (private collection, Dr Gabriela Florenţa Dumitrescu).***

The structure and function of the amniotic membrane have been analyzed by various authors so far, who investigated especially the features of the pluripotent cells of the amniotic membrane, which allow this tissue to be a very good source of grafts. The amniotic membrane has anti-inflammatory, anti-bacterial and immunological features, as well as anti-angiogenic and anti-apoptotic features [2].

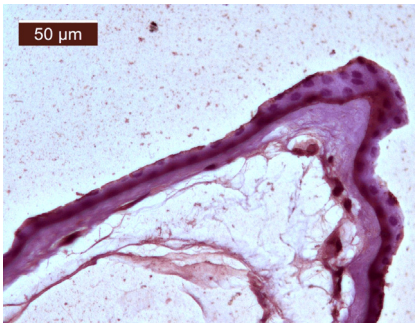

Figure 6 – ***Amniotic membrane: epithelial cell express CK AE1/AE3 (Immunostaining, anti-CK AE1/AE3 antibody, ×400) (private collection, Dr Gabriela Florenţa Dumitrescu).***

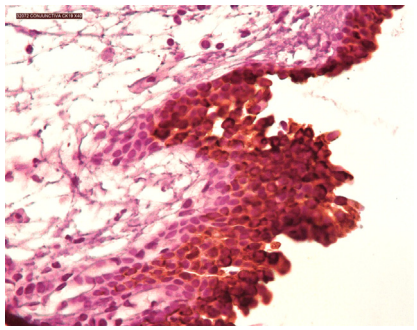

Figure 7 – ***Conjunctiva: epithelial cells shows immunopositivity for CK 19 (Immunostaining, anti-CK 19 antibody, ×400) (private collection, Dr Gabriela Florenţa Dumitrescu).***

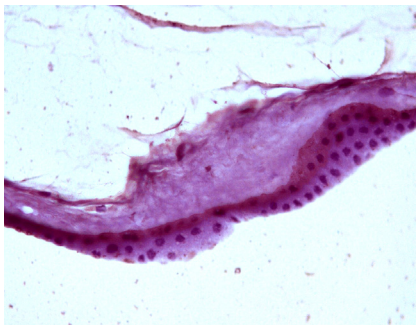

Figure 8 – ***Amniotic membrane: epithelial cells express immunopositivity for CK 19 (Immunostaining, anti-CK 19 antibody, ×400).***

Amniotic membrane procurement, processing and preservation for ocular surface

According to Kim & Tseng [7, 25, 26], the placenta, obtained under strict aseptic conditions, is rinsed using balanced salt solution containing a cocktail of antibiotics (50 µg/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin and 2.5 µg/ml amphotericin B) under sterile conditions. The amnion is separated from the chorion by blunt dissection. The separated membranes are cut in different sizes and placed on nitrocellulose paper strips with the epithelial side up. Dulbecco's Modified Eagles Medium (DMEM)/glycerol (1:1) is used for cryopreservation and the tissues are frozen at -80^{0}C until further use [25, 27–30]. Amnion stored in 50–85% glycerol is reliable and effective for over a year, with the additional advantage of its antibacterial properties [25, 31]. Both fresh and preserved AM have been found to be equally effective when transplanted on to the ocular surface [11, 32] (Figure 9, a–e).

Mechanism of amniotic membrane effects in ocular reconstruction

The membrane may be used to facilitate cornea reepithelialization in case of cornea defects and stromal ulceration [33–35], and has four main effects: (*1*)

facilitation of epithelial cell migration [36, 37]; (*2*) reinforcement of basal epithelial cell adhesion [38–40]; (*3*) promotion of epithelial cell differentiation [41–43]; and (*4*) prevention of apoptosis [44, 45].

The basement side of the membrane is an ideal substrate for supporting the growth of epithelial progenitor cells by prolonging their life span and maintaining their clonogenicity [46].

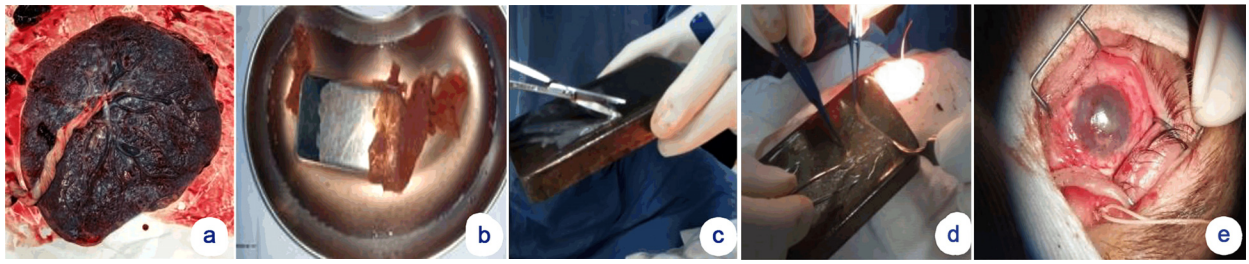

Figure 9 – ***The preparation of fresh amniotic membrane (AM): (a) AM is the inner most layer of the placenta (private collection, Dr Alexandru Cărăuleanu); (b–d) The membrane is trimmed to fit the size of the underlying defect; (e) AM is sutured to the ocular surface (private collection, Dr Claudia Florida Costea).***

The stromal component of the amniotic membrane includes a unique matrix component which suppresses TGF-β, the proliferation and differentiation of myofibroblasts, of normal human corneal and limbal fibroblasts [46, 47] and of normal conjunctival fibroblasts and pterygium body fibroblasts [46, 48]. This action explains why an amniotic membrane graft reduces scars during conjunctiva surface reconstruction [46, 49, 50], prevents recurrent scarring after pterygium removal [46, 51–55] and reduces corneal haze following phototherapeutic and photorefractive keratectomy [56, 57].

The anti-inflammatory effects of the AM are determined by the inhibition of the expression of cytokines from the damaged ocular surface, *e.g.*, interleukin (IL)-1α, IL-2, IL-8 are key regulators of the inflammatory response [58], interferon-γ, tumor necrosis factor-β, basic fibroblast growth factor and platelet derived growth factor [59]. Shimmura *et al.* [60] also demonstrated a more mechanical effect by showing that inflammatory cells get trapped and undergo apoptosis in the AM matrix.

Another characteristic of AM described in literature is the production of several potent anti-angiogenic chemicals including thrombospondin-1, endostatin and all four tissue inhibitors of metalloproteases (TIMP-1, -2, -3 and -4) [11, 61].

Antibacterial effects have been demonstrated against both Gram-positive cocci including streptococci and *Staphylococcus aureus* as well as Gram-negative bacilli including *Escherichia coli* and *Pseudomonas aeruginosa* [11, 62, 63], due to the occurrence of several antimicrobial factors in the amniotic fluid including bactricidin, betalysin, lysozyme, transferrin and 7S immunoglobulin [11, 64, 65].

Another important characteristic of the human AM is a lack of expression of the major histocompatibility antigens HLA-A, -B, or DR antigens [11, 66, 67], which allows amniotic membrane grafts in the eyeball.

Clinical ophthalmic indications

AM graft enhances growth and differentiation of conjunctival epithelial cells [40, 68] and is reported to inhibit subconjunctival scar tissue formation [47, 68] and therefore it is considered to be a favorable substrate for ocular surface reconstruction [20, 33, 49–51, 68–72].

Preserved or fresh amniotic membrane is used in the management of persistent epithelial defects in case of herpes infections, autoimmune diseases, shield ulcer, infectious keratitis [68].

It is also used as temporary biological bandage/patch. It is primarily aimed to suppress acute or chronic host tissue inflammation caused by diseases or surgery so as to promote healing with minimal scarring. AM can be sutured as a bandage (lens), dressing or patch to cover both healthy host tissue and the damage site in order to allow the healing of underneath host epithelium [11].

The use of AM as conjunctival graft for conjunctiva surface reconstruction is preferred in order to restore normal stroma and provide a healthy basement membrane for renewed epithelial proliferation and differentiation [46]. In addition to the clinical indications mentioned by Bouchard & John (2004) [73] and that could be found in Table 1, AM may be a better alternative than mucous membrane graft in plastic correction of lid abnormality and eye socket reconstruction [46]. Also, AM has been used as biological dressing in skin graft donor site in the treatment of skin burns [74], like other therapeutic methods, with an anti-inflammatory and tegument regeneration effect [75]. Moreover, AM is used for cornea surface reconstruction and as graft in case of persistent corneal ulcers of different etiologies [34–36, 54], as well as in band keratopathy [34].

Table 1 – ***Indications of AMT (according to Bouchard & John, 2004 [73])***

AMT in the presence of stem cell deficiency	Chemical ocular injuries
AMT in the absence of stem cell deficiency	Epithelial cornea defects Corneal/sclera-corneal ulcers Bullous keratopathy
AMT for conjunctiva reconstruction	Pterygium Conjunctival chalasis OSSN Limbal dermoid Symblepharon Conjunctiva lesions Leaking blebs
AMT in ocular cicatricial diseases	Toxic epidermal necrolysis Ocular cicatricial pemphigoid Oculopalpebral and reconstructive surgery
Other indications of AM use	Stem cell cultures

AMT: Amniotic membrane transplantation; OSSN: Ocular surface squamous neoplasia.

When a limbal deficiency is diagnosed, AM transplantation is designed to restore the damaged limbal environment, and in case of limbal stem cell transplantation it is aimed to restore the limbal stem cell population [20, 68, 69].

Advantages and disadvantages of amniotic membrane grafting

The thick basement membrane of the amniotic membrane facilitates epithelial cell migration and reinforces the adhesion of basal epithelial cells causing rapid epithelization. Also, it plays an important role in epithelial differentiation and prevents epithelial apoptosis [68].

Different histopathological tests have shown that the integration of the amniotic membrane graft [76] in the host cornea can be superficial, intrastromal, intraepithelial or subepithelial. This generates less vascularization during the healing process and it is done more easily, which cosmetic results that do not impair on the conjunctiva and helps to achieve corneal transparency thus enhancing visual acuity [68].

The disadvantages of the AM use are the possible total loss of limbal epithelial stem cells or of conjunctival epithelial stem cells [46]. Also, it cannot be used for eye surface reconstruction in case of severe aqueous tear deficiency, diffuse keratinization [77], absence of blinking in severe neurotrophic state and stromal ischemia [46].

Conclusions

The AM is a biologically-derived material suitable for eye surface reconstruction, since its histological structure is somewhat similar to that of the conjunctiva. Furthermore, its procurement, processing and preservation are relatively easy and after transplantation, there are no histocompatibility problems. The indications of its use in ophthalmological surgery are numerous, ranging from pterygium to neoplasia. Last but not least, the very good cosmetic results and visual acuity improvement are worth mentioning.

Conflict of interests

The authors do not have a financial interest/ arrangement or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of the manuscript.

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Corresponding author
Claudia Florida Costea, Assistant Professor, MD, PhD, Department of Ophthalmology, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iaşi, Romania; Phone +40744–972 648, e-mail: costea10@yahoo.com

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