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HYPOPHARYNGEAL AND CERVICAL ESOPHAGEAL CANCER

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Abstract 3-10% from the esophageal carcinomas invade the cervical part of the esophagus. The anatomy, treatment and prognosis of the cervical esophagus cancer are intermediary, among the hypopharyngeal and esophageal neoplasms. Following total or partial pharyngolaryngoesophagectomies a proper reconstructive technique should be adopted. Various techniques have been reported, starting with musculocutaneous flaps and finishing with colonic transposition or syntetic prothesis for pharyngoesophageal reconstruction. Total esophagectomy is indicated in the case of hypopharyngeal tumours invading the upper esophagus. The authors expose the current experience in approaching the total circular pharyngolaryngectomies, completed by total / partial esophagectomy. Of considerable importance is considered the recognition of this modern concept of interdisciplinary approach of hypopharynx and esophagus tumours, between the ENT surgeon and general / thoracic surgeon. The surgical approach, for these patients, might be considered, wright from the start, paleative and the optimum reconstruction must preserve the life quality during the survival period.

Key words: hypopharynx, cervical esophagus, cancer, multimodal treatment

Hypopharyngeal and cervical esophageal cancer represents, in majority of the cases, an aggressive spinocellular carcinoma, with a high rate of metastases, growing locally or in a distant focus. Oftentimes within the ENT oncological pathology we note tumours that affect both the hypopharynx and the cervical esophagus.

The presence of a hypopharyngeal carcinoma, invading the upper esophagus or of a cervical esophageal cancer arouse particular problems related to the surgical means of approach.

There are very few cases in which the hypopharyngeal neoplastic lesions are detected in the earliest stages of the disease, therefore the surgical treatment with complete preservation of the larynx is not achievable. At the same time, the upper esophagus infiltration requires a total esophagectomy, meaning an interdisciplinary approach.

The hypopharynx (laryngo-pharynx) communicates with the esophagus at its lower part, corresponding posteriorly to the C4-C6 vertebres.

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In reference to the anatomical localizations of the hypopharyngeal carcinoma we identify the posterior wall, the lateral walls, the pyriform sinuses and the retrocricoid pharynx.

Regarding the surgical approach, the retrocricoid tumors with upper esophagus infiltration bring up particular difficulties, recognizing the fact that the anterior wall of the retrocricoid region is called ‘party wall’.

The lateral and posterior pharyngeal walls are circumscribed by the constrictor muscles. Hypopharyngeal walls examination is performed by indirect laryngoscopy, but this procedure has no value in examining the retrocricoid area. The laryngeal part of the pharynx has anatomical rapport with thyroid gland lobes, commune carotid artery and its bifurcation branches, vagus nerve and recurrent nerves.

The cervical esophagus lays between the upper esophagus and the superior aperture of thorax(T2-T3).

The upper part of the esophagus corresponds to the pharyngo-esophageal junction, between the retroarytenoid area and the cervical esophagus.

The cervical esophagus has an average length of 4-5 cm, its lower limit being situated at approximately 18 cm from the dental arcade.

The cervical esophagus has posterior rapports with vertebral bodies and its anterior rapports are with trachea, thyroid gland, recurrent laryngeal nerve, inferior thyroid artery.

The vascularization is supplied by branches from inferior thyroid artery and ascending branches from the thoracic part.

Lymphatic drainage is performed at the level of recurrent lymph nodes (VI bis), inferior jugulo-carotid lymph nodes, paratracheal lymph nodes, superior mediastinal lymph nodes.

As frequency, esophageal cancer is situated on the 8-th position, 412000 new cases being recorded in 2000 (1, 2).

The esophageal carcinoma incidence is related to the geographical area and the presence of risk factors.

Within epidemiological studies the cervical esophageal cancer cannot be always individualized, appearing as 3-15% from the amount of esophageal carcinomas.

The risk factors are similar to the hypopharyngeal cancer risk factors: alcohol (80%), smoking (65%), eating habits, Plummer-Vinson syndrome, family history. Evidence based medicine has shown an earlier age of hypopharyngeal and cervical cancer occurrence in persons with family history of cancer.

There is not a well established correspondence between the life style and the family history of pharyngoesophageal carcinoma.

Regarding the histopathology, the pharyngoesophageal neoplasms consist in general an spino-cellular carcinomas.

Within ‘Coltea’ ENT clinic cases evidence, the histopathological results have shown a prevalence for well differentiated carcinomas: 54% well-differentiated invasive spino-cellular carcinomas, 28% mild-differentiated carcinoma, 82% of mild and well-differentiated) and 18% of poor-differentiated carcinomas.

The hypopharyngeal posterior wall tumours tend to remain on the hypopharyngeal posterior wall.

The hypopharyngeal lateral wall tumours, in late stages, have the tendency to extend laterally, through the pharyngeal constrictor, invading the parapharyngeal muscles, the 9-th and 10-th nerves, the cervical sympathetic nerve, the ipsilateral thyroid lobe. Secondly they infiltrate the pharyngoepiglottic folds, the valleculae, anterior and lateral walls of pyriform sinus. Pyriform sinus tumours invade the larynx, immobilizing the arytenoids and vocal cords, then the retrocricoid area, the contralateral pyriform sinus and, in the late stages of the disease, the cervical esophagus. The tumours of the retrocricoid region infiltrate the pharyngeal wall in circle and sometimes they invade early the pyriform sinus apex.

Lymphatic dissemination follows two paths: first, the jugulo-carotid lymph nodes and second, the spinal nodes. The jugulo-digastric nodes are the most frequent affected. Wang accounts of 55% positive lymph nodes in hypopharyngeal posterior wall carcinomas and 10% out of 55% appear as bilateral lymph nodes.

On admission 75% of patients with pyriform sinus cancer present clinical lymphadenopathies, 10% bilateral, and Ogura reports subclinical invasion in 62% of cases.

Cervical esophageal tumours disseminate by local, lymphatic and vascular invasion. Professor Dan Gavriliu supported the fact that the lymph nets eyelets within the mucous membrane and muscular layer have an elongated (elliptical)
shape, explaining in this manner the malignant tumours tendency to intramural metastasis, away from the macroscopic lesion’s site. This theory explains the performance of total pharyngolaryngo-esophagectomy in the cases of hypopharyngeal and cervical esophageal cancer. According to Cordos the macroscopic extension away from the tumour does not exceed 4 cm.

TNM Classification of cervical esophageal cancer:
- **T1** - tumour invasion, limited to the submucous membrane;
- **T2** - tumour invasion, limited to the muscular membrane;
- **T3** - tumour invades the external membrane;
- **T4** - tumour invasion to the neighbouring structures
- **No** - no lymph nodes invasion
- **N1** - regional lymph nodes invasion (paraesophageal, mediastinal and abdominal lymph nodes, the 2nd anatomical station)
- **Mo** - no distant metastases
- **M1LYN** - supraclavicular and phrenic lymph nodes metastases (the 3rd anatomical station)
- **M1** - positive visceral metastases

The symptomatology in esophageal tumours is dominated by dysphagia, initially for solids and then also for liquids, with nutritional status alteration of the patient. The patient’s general status is stable in the beginning but changes progressively. The patient complains of a sensation of retrosternal pressure, associated with hypersalivation, regurgitation, eructation. Retrosternal, dorsal (interscapulovertebral) and epigastric pains appear in advanced stages. Upper gastrointestinal bleedings recur in ulcerated tumours. Dysphonia (recurrent nerve paralysis), Claude-Bernard-Horner (lesion of the cervical sympathetic plexus), supraclavicular lymphadenopathies, distant metastases (liver, lungs, bone, brain) may arise in the course of the disease.

The dysphagia, sensation of a foreign body, otalgia, hypersalivation with blood marks, dysphonia are specific to the hypopharyngeal lateral wall and pyriform sinus tumours. The lesions can be diagnosed by indirect laryngoscopy, which is ineffective in the case of pyriform sinus apex and retrocricoid tumours. Retrocricoid neoplasms displace the larynx anteriorly.

Lab tests reveal the presence of anaemia, hypoproteinemia, modified liver function tests, hypercalcemia in 15% of patients. Citological screening is of value in the case of populations with high risk. The paraclinical diagnosis is established by standard and contrast X-rays, CT, MRI. Endoscopic exploration (suspended laryngoscopy, esophagoscopy, bronchoscopy) and intraluminal ultrasonography are essential in diagnosing and staging of tumours. The final diagnosis is dictated by the histopathological and immunohistochemical exam.

The treatment of hypopharyngeal and cervical esophageal cancer arouses particular problems being a multimodal treatment that involves collaboration between the ENT surgeon, radiotherapist, chemotherapist, nutritionist and psychologist. In reference to the surgical approach in most of the hypopharyngeal and cervical esophageal neoplasms the larynx preservation is not achievable. In the case of posterior wall tumours, measuring less than 3-4 cm, it is possible to perform partial pharyngectomy and reconstruction with mobile skin grafts (Seifert procedure) or with mobile radial flap. Total circular pharyngectomy (Paul Andre type) followed by partial or total esophagectomy is performed in majority of cases of hypopharyngeal and cervical esophageal invasion. There are different suppositions regarding the extent of the esophageal resection when the pharyngoesophageal junction is invaded. Regardless of the method of surgical approach the reconstruction of the gastrointestinal tube continuity appear as the surgeon’s bid. The ideal reconstruction has to have the following features:

1. is to be performed in one step;
2. low cost;
3. reduced mortality;
4. reduced mobility;
5. short period of hospitalization;
6. post-op radiotherapy tolerance.

The success of the reconstruction is conditioned by:
- the extent of the pharyngeal deficiency;
- the presence of larynx;
A defect is considered to be circumferential when it surpasses 70% of the pharynx circumference or when the post resection remaining mucosa measures less than 2 cm. In the case of caudal lesions, when the esophagus is invaded and the resection margin is in the mediastinum, the most appropriate reconstructive techniques consist of gastric ascension, colonic transposition and possible, synthetic prosthesis of big sizes.

In the case of cephalic circumscribed lesions, when the resection margin is within the cephalic area, of an important value are mobile fasciocutaneous flaps, musculo-cutaneous flaps, jejunal auto-graft or pharyngoesophageal prosthesis.

In ‘Coltea’ ENT Clinic from 2000, after circular pharyngolaryngectomies, Professor ENT Surgeon Cristian Popescu (3) achieves the pharyngoesophageal continuity using Montgomery protheses (21 cases).

Before these surgical treatment procedures is has been tried in 3 cases the reconstruction of the pharyngoesophageal continuity using prothesis made from various synthetics materials (dacron, plastic), but they have been rejected by the patients body.

The pharyngoesophageal prothesis are fabricated from silicon, they exist in different sizes and can be radioopaque or radiotranslucent. At its tubular part the prothesis presents two globular areas, which provide a better stability within the focus and maintain the saliva drainage, regardless the head or neck position. The use of Montgomery prothesis is done:

- to improve the life quality;
- as an intermediary stage until the reconstruction with free flaps.

Their superior edge is sutured at the tongue base and oro/ hypopharynx. The inferior extremity is introduced in the remaining esophagus.

- the inferior extension of the tumor.
In order to provide a proper stability within the focus it is very important to preserve and reconstruct the prelaryngeal muscles. The radiological and endoscopical examinations allow to verify the prothesis position and permeability. The average hospitalization time period is 2-3 weeks and oral alimentation can be re-attained after 10-14 days.

The nasogastric tube, introduced intraoperatory through the Montgomery prothesis lumen, is suppressed following the methylene blue test performance.

In the case of total esophagectomy an interdisciplinary approach is required, involving the General Surgeon and ENT Surgeon, for a reconstruction to be achieved, and regurally gastric ascension and colonic transposition are used (4, 5, 6, 7, 8, 9).

Pharyngoesophageal continuity reconstructions, using free jejunal loop or radial flap, impose collaboration with a surgeon trained in reconstructive microsurgery techniques.

Esophageal resection without thoracotomy is indicated in hypopharyngeal and cervical esophageal carcinoma, where the lymph nodes resection may be performed under visual control. It is possible to perform radical cervical lymph nodes dissection on one side and modified radical on the other side, therefore on the fascia paraevertebralis only the carotid artery, jugular vein and vagus nerves remain.

Surgical treatment is followed by radiotherapy. The dose is related to the histopathological result, tumor location, resection grade and used reconstructive technique. Thus, in the case of reconstruction using Montgomery esophageal prothesis, the tolerance to radiotherapy is similar to the one corresponding to other reconstruction methods. The average doses administered to patients, beneficiaries of Montgomery prothesis, were 65 Gy, in comparison with 40 Gy, in case of reconstruction using stomach (gastric pull-up), 70 Gy in musculocutaneous flaps reconstruction and 60 Gy in reconstruction using jejunum. Irradiation technique is complex, using anterior and anterior oblique fields and the irradiation volume include the primary lesion and the lymph nodes areas (cervical, paratracheal and paraesophageal within the superior mediastinum) (10).

In the case of hypopharyngeal and cervical esophageal cancer simultaneous radiochemotherapy is indicated for tumours with high risk of local and regional recurrence. Preoperative radiotherapy (20-40 Gy) and chemotherapy have not demonstrated favorable results regarding survival rate.

In cervical esophageal cancer the photodynamic therapy may be used both as a curative method and as a palliative method (alleviates the dysphagia).

Among other palliative procedures we note: tracheostomy, gastrostomy, esophageal dilations and stents, deobstructions using Laser YAG or using bipolar cautery, radiochemotherapy in inoperative tumours.

Clinical nutrition must be initiated, following evaluation of patient’s nutritional status (BMI, NRS2000), from the preoperative period and extended intra and postoperative, depending on the caloric demands and the patient’s biochemical status. Enteral nutrition, using nasogastric/jejunal tube or gastrostomy tube, with standardized nutri-
Figure 6, 7. Free transposition of jejunal loop, following total pharyngolaryngoesophagectomy (from the iconography of Prof. Dr. C. Ciuce)

Figure 8, 9, 10, 11. Pharyngoesophageal reconstruction using Montgomery prothesis (from the iconography of Prof. Dr. C.R. Popescu)
tional solutions, according to ESPEN2006 recom-
mendations, is part of the complex treatment of
hypopharyngeal and cervical esophageal cancer
(10,11,12,13,14,15,16,17).

Regardless of modern techniques of diagnosis
and staging, of various surgical procedures of
ablation and reconstruction, of radiotherapy and
chemotherapy, the prognosis for patients with
hypopharyngeal and cervical esophageal cancer
is modest.

Of an important value is the recognition of this
modern interdisciplinary concept of approaching
the cervical esophageal tumours, involving the ENT
surgeon, general/ thoracic surgeon, radiochemo-
therapist, nutritionist, psychologist (18,). In the case
of these patients, the surgery may be considered
from the beginning as palliative and the optimal
reconstruction must preserve the quality of life
during the survival period.

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CLINICAL AND PATHOLOGICAL FEATURES OF ESOPHAGEAL GRANULAR CELL TUMOR - CASE REPORT OF SEVEN PATIENTS

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Abstract Granular cell tumors (first described by Abrikossoff in 1926) are generally benign lesions that appear most frequently in esophagus. We report a number of seven cases discovered incidentally at routine upper digestive endoscopy. Tumor samples from biopsies were analyzed using hematoxilin and eosin stain, PAS stain and immunohistochemistry for S-100 protein. The symptoms of the patients are nonspecific: epigastric pain, abdominal discomfort, bleeding. The lesions appears like small (diameter between 4-12 mm), yellow-white, firm lesions in the lower esophagus. The histological sections on biopsy revealed sheets of polygonal cells, without clear cellular limits, a granular cytoplasm and centrally-located small, round, dark nuclei. These lesions are not circumscribed and come into intimate contact with the overlying surface squamous epithelium. The Periodic Acid-Schiff positive cytoplasm contained abundant eosinophilic granules. Immunohistochemically the tumor cells had diffuse, strong positive cytoplasmic staining with antibodies to S-100 protein. The morphologic findings and immunohistochemical staining pattern supported a diagnosis of granular cell tumor. The granular cell tumors (Abrikossoff tumors) of the esophagus are rare, benign, sessile, yellow-white tumors discovered incidentally at the upper digestive endoscopy. The polygonal cell tumors have a granular periodic acid-Schiff positive cytoplasm and a diffuse immunoreactivity for S-100 protein, a marker which supports neural origin of these lesions.

Key words: granular cell tumor, esophagus, S-100

Introduction

Over 200 of granular cell tumors (Abrikossoff tumors) were discovered in esophagus¹ and a lot in other various organ systems but frequently involve areas that include the tongue, skin, breast and digestive tract. Abrikossoff first described (cited by David O et al)² this tumor in 1926. The origin of these tumors was believed to be from skeletal muscle because they were found insinuated within muscle. For many years, these lesions were referred to as granular cell myoblastoma. The histogenesis of this lesion is controversial, but immunohistological and ultrastructural studies supporting a perineural cell origin.

Granular cell tumors are rare benign tumors and sometimes malignant (1-3%)³,⁴, discovered in every segment of digestive tract, the commonest site being esophageal level. In 11% of cases it was described an association with

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other types of malignant tumors in the digestive or respiratory tract. Usually are solitary lesions but are described also multiple neoplasm.

The most of granular cell tumors are developed submucosal or subepithelial with the epithelium often showing hyperplasia. Usually, Abrikossoff tumors are discovered incidentally during the upper gastrointestinal endoscopy. The macroscopic features are sessile, yellow or yellow-white firm nodules.

Sometimes could be present unspecific symptoms: dysphagia, bleeding or abdominal discomfort. Tumors are positive for S-100 protein, neuron specific enolase and myelin basic protein but were described positive cases for other markers: vimentin, CD68, protein gene product 9.5 (PGP 9.5).

Methods

Seven patients were presented at the hospital for nonspecific gastrointestinal symptoms:
• 2 patients with epigastric pain and pyrosis
• 3 patient with abdominal discomfort
• 1 patient with bleeding
• 1 patient for staging of chronic hepatic disease

Upper digestive endoscopy discovered small solitary lesions on the lower esophagus wall or at Z line level. Every nodular lesion was biopsied and samples were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned to 4 µm thick sections and stained with hematoxylin and eosin and PAS (periodic acid-Schiff).

Immunoperoxidase stain was performed on deparaffinized sections from the tissue block. An avidin-biotin-peroxidase complex method was used to detect the tissue antigen with S-100 antibodies (dilution 1:500).

Results

Submucosal tumors are frequent incidental findings during endoscopy, although definitive diagnosis based on histological confirmation presents some difficulties as they often only contain the normal appearing overlying mucosa.

In our report, all seven tumors are discovered incidentally on routine upper digestive endoscopy. All lesions were solitary, with diameter between 4-12 mm and localized in the lower esophagus (Table 1).

Macroscopic, all seven tumors were sessile, yellow-white nodular lesions (Figure 1, 2, 3), with a firm consistency on biopsy.

Table 1. Dimensions and localization of esophageal tumors

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor dimension (diameter - mm)</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>46</td>
<td>F</td>
<td>5</td>
<td>Z line</td>
</tr>
<tr>
<td>2.</td>
<td>43</td>
<td>M</td>
<td>12</td>
<td>40 cm from incisor teeth</td>
</tr>
<tr>
<td>3.</td>
<td>34</td>
<td>M</td>
<td>4</td>
<td>Z line</td>
</tr>
<tr>
<td>4.</td>
<td>46</td>
<td>M</td>
<td>7</td>
<td>30 cm from incisor teeth</td>
</tr>
<tr>
<td>5.</td>
<td>48</td>
<td>F</td>
<td>5</td>
<td>35 cm from incisor teeth</td>
</tr>
<tr>
<td>6.</td>
<td>57</td>
<td>M</td>
<td>5</td>
<td>30 cm from incisor teeth</td>
</tr>
<tr>
<td>7.</td>
<td>47</td>
<td>M</td>
<td>10</td>
<td>30 cm from incisor teeth</td>
</tr>
</tbody>
</table>

Figure 1. Macroscopic image of a 12 mm yellow-white nodular lesion
The histological sections revealed sheets of polygonal cells (Figure 4), without clear cellular limits. The cells had a low N/C ratio with centrally-located small, round, dark nuclei (Figure 5). The tumors are not circumscribed and come into intimate contact with the overlying surface squamous epithelium. The PAS positive cytoplasm contained abundant eosinophilic granules and occasional eosinophilic cytoplasmic globules (Figure 6).

An immunoperoxidase stain with antibodies to S-100 protein was performed on a representative section of the formalin-fixed, paraffin embedded tumor. The tumor cells demonstrated diffuse, strong positive cytoplasm staining with antibodies to S-100 protein (Figure 7).
Clinical and pathological features of esophageal granular cell tumor - case report of seven patients

The morphologic findings and immunohistochemical staining pattern supported a diagnosis of granular cell tumor.

Discussions

Esophageal granular cell tumors are benign lesions, frequently discovered incidentally at endoscopy and long-term follow-up revealed no evidence of tumor progression. Malignant granular cell tumors are extremely rare, estimated to 1-2% of all cases discovered. Fahnburg-Smith et al proposed in 1998, some histological criteria to define malignant granular cell tumors: spindling, vesicular nuclei with prominent nucleoli, increased mitotic activity (>2 mitosis/10 high power fields at 200 magnification), a high N/C ratio, nuclear pleomorphism and the presence of necrosis. They suggested that a tumor who satisfies at least three of these criteria could be histologically classified as malignant. Wieczorek et al proposed little different criteria of malignancy: hyperchromasia, coarse chromatin, increased N/C ratio, nuclear pleomorphism, vesicular nuclei with enlarged nucleoli and spindle cell morphology; mitoses were present in malignant granular cell tumors and absent from all benign granular cell tumors.

Immunocytochemically, the granular cells are positive for S-100 protein, vimentin, NSE, keratan sulfate, CD57, CD68. The tumor is immunocytochemically negative for endocrine and myogenic markers, for CD34 and c-kit, markers that could be used to differentiate the granular cell tumor from an endocrine, myogenic or stromal gastrointestinal tumor.

In our cases we did not find histological features of malignancy and all the cases showed positive cytoplasm immunoreactivity for S-100 protein.

Asymptomatic, smaller lesions require observation only. Larger, symptomatic lesions can be treated with local surgical excision. Endoscopic ultrasound can provide additional information on the layer of origin and tumor extension. When deciding whether to proceed with surgical or endoscopic resection, endosonography plays a key role in establishing whether the tumor is confined to the submucosa. Tacheuchi et al proposed that strip biopsy to be considered as a viable alternative treatment for esophageal granular cell tumor, depending on the histologic character, tumor size, and depth of tumor infiltration. Tumors confined within the hyperechoic layer of the submucosa could be successfully treated by endoscopical excision without complications or signs of relapse during the follow-up period.

Our patients have small esophageal lesions with one case exception - 12 mm diameter - that was endoscopically excised. The follow up (six months - one year) did not reveal a local recidive or increase in dimensions of the esophageal lesions.

Conclusions

The most of the granular cell tumors of the esophagus are rare, benign tumors discovered incidentally at the upper digestive endoscopy. They have a protrusion, sessile feature, with a yellow-white color and firm consistency at the biopsy. Abrikossoff tumors are positive for S-100 protein, a marker which supports neural origin of these lesions.

Smaller and asymptomatic lesions require only observation but when the patient is symptomatic or the tumor is greater than 1 cm or has atypical histological features, the surgical or endoscopical resection is necessary, depending on the depth of tumor infiltration.
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MOLECULAR FACTORS IN CUTANEOUS MALIGNANT MELANOMAS AGGRESSIVITY

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Abstract
Malignant transformation of melanocytes is a multistep process characterized by distinct histopathological stages. Many studies in molecular pathology indicated that bcl-2, ki-67 and p-53 immunoreactivity can be use to detect cases with unfavourable prognosis. Aim: To study the expression of bcl-2, ki-67 and p-53 in 50 cases of coutaneous malignant melanomas (15 cases in situ and 35 cases invasive melanomas) as a prognostic factors. Materials and methods: Our study included 50 cases of coutaneous malignant melanomas. H&E and paraffin section immunostaining for bcl-2, ki-57 and p-53 were performed in all cases. (IHC study was carried on formalin fixed, paraffin-embeded tissue using the avidin-biotin peroxidase complex). Results: p-53 expression was positive in most all cases, values between 2%-60%; bcl-2 expression was positive in 93% cases; ki-67 expression was positive in most cases, values between 4%-72%. Discussion and conclusion: Our frequency detection is not related to histopathological tipe, tumor location, size, depth, ulceration, mitotic activity and growth phase. Most lesions are p-53 expression higher (24% cases) and was associated with poor survival; bcl-2 expression was higher in 93% cases and was associated with good prognosis. Although ki-67 expression was higher in 35% cases, it is not related with prognosis. It was suggested that p-53 and bcl-2 expression could be useful in predicting the biological behaviour.

Key words: cutaneous malignant melanoma, agressivity, mollecular factors

Introduction
Melanomas are malignant tumours deriving from the transformation and proliferation of melanocytes which normally reside in the basal cell layer of the epidermis. In the past was considered as a tumour with low frequency, but the recent scientific studies have shown a rate of increase in almost every country and a descent of the age that appears.

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Although the modifications of cutaneous pigmentation are very accessible, it has been shown that the diagnosis of the malignant melanoma is given in aggressive stages. The mortality in malignant melanoma is ascendent because it exists a high, relative resistance at the current oncological terapies.

**Material and Method**

From the many cases of cutaneous malignant melanomas diagnosed in the Pathology Department of the Dermatology Clinic of Colentina Hospital, we selected 50 patients that presented lentigo maligna (5 cases), lentigo maligna melanoma (4 cases), superficial spreading melanoma (5 cases), superficial spreading melanoma with vertical growth phase (20 cases), nodular malignant melanoma (14 cases), acral lentiginous melanoma (2 cases).

The materials that were used were represented by surgery pieces (cutaneous tumours excised with oncological area around them). These were manufactured for the histopathological examination (formalin fixed, dehydrated, then included in paraffin and the multiple sections were stained with HE).

The histopathological examination has shown the following criterions: the histogenetic type, the depth of the tumoral invasion (Clark) and the maximum thickness of the tumour (Breslow), the presence of the ulcerations, mitotic rate, the tumoral profile (plans or polypoid lesions), the presence of the melanin and tumor-infiltrating lymphocytes.

Immunostaining was performed on new 5-micron thick paraffin sections; after deparaffination and hydrating, the modified Avidin-Biotin-Peroxidase complex method of Hsu et al. (Bussolati and Gugliotta, 1983) was applied. The monoclonal antibodies and the polyclonal sera were used (table 1).

### Results

In cases we’ve studied resulted that is a slight female preponderance and the age group that were affected are the 6-th, 7-th and 5-th decades. Although tumor thickness/Breslow and the levels of invasion suggested by Clark, their values were increased. (fig. 1)

The upper dermis which almost always shows severe elastic solaregeneration, contains numerous melanophages and pronounced inflammatory infiltrate.

Lentigo malignant melanoma is defined like a cutaneous malignant melanoma with intraepidermical lentiginous component. For these cases the histopathological examination shows atrophic epidermis with more pronounced increase in the concentration of basal melanocytes; this cells are round with atypical nucleus and then they come to lie in contiguity with one another, and their number increase many of them are elongated in vertical growth phase. The melanocytic proliferation is found also in hair follicle epithelium, in basal cells layer. The stromal reaction is classical represented by lymphocytic inflammatory infiltrate with several melanophages that look like some ‘balls of coal’. In the vertical growth zones we ascertain an increasing melanocytes concentration like some acromic, spindle-cell melanocytes nests; a few mitoses.

Superficial spreading melanoma: is a cutaneous malignant melanoma with intraepidermical pagetoid component. His evolution requires a radial growth phase and a vertical growth phase (fig. 3). Histopathological the lesion is asymmetrical; the epidermis is irregularly thickened and thinned; many large, rounded melanocytes lie in nests or singly and are scattered in a pagetoid pattern throughout the epidermis. The nests tend to vary in size and shape and sometimes become confluent (fig. 2); the cells have atypical, hyperchromatic nuclei with conspicuous nuclecleoli and

### Table 1.

<table>
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<tr>
<th>Antibody</th>
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<tbody>
<tr>
<td>bcl-2</td>
<td>bcl-2 oncoprotein</td>
<td>DAKO, Glostrup</td>
<td>1:40</td>
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<td>p-53</td>
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<td>Biogenex</td>
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<tr>
<td>ki-67</td>
<td>antigen of the celular proliferation</td>
<td>Biogenex</td>
<td>1:1</td>
<td>MIB-1</td>
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the cytoplasm containing melanin like 'dusty' particles. Usual the lymphocytic infiltrate is dense and containing melanophages.

The melanocitary population from tumorigenic vertical growth phase has polyclonal aspect (from the beginning may be monomorphous). The tumoral cells are big, atypical, rounded, spindle shape or nevocytic like, with mitotical activity.

Nodular malignant melanoma or tumorigenic melanoma is defined like a melanoma without radial growth phase. Histopathological the tumor mass tend to be symetrical and the epidermis overlyind expands marked elongation of the rete ridges.

Usually the tumor has ulcerated epidermis and presented in surface lentigious or pagetoid aspects. Melanoma cells are mixed pattern: epithelioid and spindle cell (Fig. 5). Epithelioid melanoma cells are large and rounded with pleomorphic, vesicular nuclei who contain eosinophilic nucleoli; mitotic figures may be numerous and sometimes abnormal; pigmentation is variable and may be minimal or abundant (Fig. 6). Sometimes we found gigant multinucleate cells, very pleomorphic, with large vesicular nuclei containing nucleoli and conspicuous, abnormal, mitotic figure. The epithelioid cells tend to lie in alveolar formation and the spindle shaped cells in irregular branching formations; the alveolar formations are surrounded by thin fibre s of collagen.

Acral lentigious melanoma: is defined like a cutaneous malignant melanoma with intraepidermical lentigious component localised in acral areas. Histopathological, is caracterised by irregu-
lar acanthosis with atypical melanocytes located in dermal - epidermal junction (Fig. 7); some tumor cells can be found in the upper layers of the epidermis, especially near areas of invasion in the centers of the lesion and others cells extending within the sweat gland epithelium. The lower reaches of the epidermis are infiltrated by large numbers of atypical melanocytes characterised by nuclear pleomorphism and hyperchromatism; the invasive tumor is often spindle cell in type and commonly elicits a desmoplastic reaction. Pigmentation is often pronounced, and results the presence of melanophages in the upper dermis and of large aggregates of melanin in the broad stratum corneum. There may be a lichenoid lymphocytic infiltrate.

**p53** (oncosuppressor gene): it has been shown to be a cellular growth and transformation supressor, by blocking cell cycle in G1 stage (when DNA can be repaired if an abnormality occurs) as well as an apoptosis stimulator (Fig. 9). P53 mutations are the most common seen genetic abnormalities respon-

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Figure 4. Nodular malignant melanoma; pleomorphic malignant melanocytes lies in nests; (HE; 20x)

Figure 5. Nodular malignant melanoma; pleomorphic malignant melanocytes lies in nests; (HE 20x)

Figure 6. Nodular malignant melanoma; pleomorphic tumoral cells with variable pigmentation; giant cell, mono-, bi- or multinucleate, small cell, epithelioid cell with vesicular nuclei and conspicuous nucleoli; (HE 40x)

Figure 7. Acral lentiginous melanoma; irregular epidermal hyperplasia; nests of atypical melanocytes in dermal-epidermal junction; fascicles of spindle-cells deep in the dermis; lymphocytic infiltrate and melanophages; (HE 10x)
Molecular factors in cutaneous malignant melanomas aggressivity

sable for human cancer development. Its actions can be suppressed either by a mutation that affects the gene reading sense (missense alteration), or by interaction with viral oncoproteins or other cells. 'Wild' p53 gene function loss determines abnormalities of cellular cicle control and DNA replications (defectuous DNA repairing), favouring the growth of certain cells and, subsequently, formation and development of tumoral process. Secondary to cellular cycle blocking in G1, p53 is accumulated in the nucleus and this can determine gene mutations. Mutated p53 has a half-life time much higher than the 'wild' gene (44 minutes versus 20 minutes). As a consequence, determination of p53 by immunohistochemistry methods shows an accumulation of the mutated variant. Because p53 is also a transcription factor, it has been suggested that the mutated variants / forms can interact with various transcription loci determining the high cellular proliferation rate characteristic for neoplasia. P53 mutations can be correlated with DNA alterations by oncogenic factors such as: UVB radiation, aflatoxine, oxidative processes. Prognostic value of p53 gene ("the guardian of the genome") is still unclear. Chen and co have shown that the frequency of mutated p53 is correlated with tumor stage and degree.

**BCL 2** (B cell lymphoma 2) is the main gene that blocks programmed cellular death (apoptosis). It is found on the mitochondrial membrane (BCL2α) or cytoplasm (BCL2β) of the progenitor cells with long life and of the epithelium being influenced by growth-factor (Fig. 8). Its role is to inhibit the apoptosis induced by growth factor and to determine continuous cell proliferation even in the absence of mitotic factors.

**Ki-67** can recognize a nuclear antigen expressed during G1, S, G2, and M of cellular cycle but absent in G0 stage (of resting). There is a correlation between increased ki-67 activity and the other aggressive markers (mitotic activity, histological stage) (Fig.10).

**Conclusions**

We observed that the expression of the immunohistochemical markers used is not correlated with the histopathological type of malignant melanoma, tumor growth, patient age or gender.

It is important to mention that p53 positivity was higher for tumors located on sun exposed body regions such as head and neck.
**P53** values in tumor cells were between 2 - 80% positivity. Sun radiation, especially UVB, could be involved, inducing p53 gene mutations. It is possible that secondary to UV exposure during childhood, epidermal melanocytes are mainly initialized and transformed. There are also other factors that promote and lead to tumor development depending on host phenotype and environment factors. A pathogenic way is characterized by abnormal expression of p53 gene associated with epidemiological markers of sun sensibility and high levels of ultraviolet sun exposure during life. A second way is characterized by an inherent tendency for focal melanocyte hyperpigmentation / proliferation such as ephelides or naevi and it is not correlated with p53 expression. The strongest expression of p53 in dysplastic naevi suggests that its overexpression could be the first step in the loss of cell cycle regulation and possible malignant transformation of melanocytes.

**Bcl-2** plays a protective role against apoptosis and was positive (either focal or diffuse) in tumor cells of all studied cases.

**Ki-67** was positive in most of the cases with values ranging between 4 and 70%.

It is important to underline that only the presence of cell proliferation markers it is not sufficient and they must be quantified in order to determine their staining markers. As a consequence, we must determine the number of cells, respectively the stained nuclei number per 1 000 cells which it is then divided to 10 in order to achieve the proliferation index. This operation should be done for several microscopic fields with high cellular density which it’s difficult to achieve by free appreciation - simple counting. The counting should be done by computer, with a microscope having an image analyzer with a McIntosh programme, called IHL. It is understandable that the results obtained by the two counting methods are different, explaining the possible error of free counting of a large number of cells, as well as of the staining.

Histopathologically the most important markers of tumor invasion remain Clark level and Breslow tumor thickness. Most studied melanomas were IV Clark level and had a tumor thickness over 1,5 mm. Occurrence of tumor ulceration had a high prognostic value (over the prognostic is unfavorable if it is higher than 6 mm). Taking into account the tumor profile, the polipoidal lesions have a less favorable evolution than those flat or en en dome. Stromal inflammatory reaction consists of band like lymphocyte infiltrates, under the lesion, with or without melanophages. When the tumor is ulcerated, in the inflammatory infiltrate can occur several plasmocytes. Inflammation is correlated with the invasion depth; when the Clark level is increased, inflammation decreases. Inflammatory reaction occurs when the tumor has one of the first three Clark levels. Neoplastic embolisations were identified in 3 cases of the study group. Regression of certain parts of the tumor has a unfavorable prognostic. We also noticed proliferated and dilated blood vessels, especially of the capillaries inside of the tumor, explaining distance malign cells dissemination and subsequently occurrence of metastasis.

Accordingly to our study results we concluded that malignant melanoma is a tumor with a high growth, having an increased invasion, an unpredictable evolution and generally with an unfavorable prognostic, except those tumors early diagnosed (I Clark level and Breslow tumor thickness less of 0,76 mm).

**References**

ETHICAL AND METHODOLOGICAL PRINCIPLES IN CONTEMPORARY SURGICAL SCIENTIFIC RESEARCH

Viorica Vidu, N. Bacalbasa

General Surgery and Liver transplantation Center, Fundeni Clinical Institute, Bucharest

Abstract In contemporary medicine, research wishes itself to be a new, reborn, academically crowned activity, characterized by tenacity, objectivity and multidisciplinarity. Medicine is research and cannot exist without research, so the researcher's part becomes essential in scientific progress. The researcher-surgeon's activity must be submitted to the ethical principles of surgical research: dignity, honesty, responsibility, efficiency, education, caution; as such, the result of his work is considered to have scientific value.

Key words: surgical research, priority, multidisciplinarity

In the early '90s a new medical branch, considered to have a future, appeared: research. For decades shadowed, Romanian scientific research wishes itself to be, nowadays, a new, reborn, academically crowned profession well represented in all medical branches in university centers.

Imposed as a necessity by the spectacular scientific progress, the researcher must be the promoter of the new values, connection between the laboratory or library scientific horizon and medical practice, thus obtaining new scientific research objectives and the means of putting them into practice.

It is unanimously accepted that medicine cannot and never has existed without research, because, 'medicine is in itself research' as considered professor Jean Bernard in his essay 'The doctor and the researcher'. In his opinion, the doctor examining a patient does a research activity in order to understand the essence of the symptoms which brought the latter for consult, an individual research, limited to an immediate objective, never the less being able to open the way for a new discovery.

Therefore, medicine is conceptually a research activity and cannot exist without research!

The great medical discoveries, which changed the destiny of mankind followed two major pathways: clinical research and fundamental (genetics, biology, biophysics, immunology) research, lately these fields becoming much closer, progress in medicine being the result of their intertwining. This aspect is the fundamental mark of contemporary research, which contributed to the appearance of researchers in each individual medical field, as an imperious necessity to combine clinical medicine and fundamental science.

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In the field of surgery this aspect materialized in remarkable progress in microsurgery, organ transplantation, oncologic surgery; new therapeutic methods such as laparoscopic and endoscopic surgery techniques emerged as a result of collaboration of surgeons and biologists, biophysicists, geneticists, engineers.

Worldwide and in Romania, liver surgery is one of the fields in which the most spectacular progress was achieved. The means of investigation became more precise and sophisticated, resection techniques were diversified and complementary treatment methods were developed for malignant tumors which led to improvement in results.

However, the most imposing success in liver surgery was the liver transplant which became a therapeutic method accepted in current medical practice, also in Romania.

As a consequence the introduction of liver transplantation in the treatment of terminal liver disease led to a new, modern, more complex interpretation of liver physiology and pathology, and also a reconsideration of attitude in the treatment of severe diseases considered so far considered hopeless.

Of a great notoriety in this field is the personality of Thomas E. Starzl, researcher and liver transplantation surgeon, who, in 1968, introduced the liver transplantation in medical practice.

Also, his scientific contribution to kidney transplantation, use of ciclosporins in immunosuppressive treatment, hepatotrophic influence of insulin, multi-organ transplantation and xenotransplant are undeniable values of contemporary surgical research, which led to the progress of medicine in a new era, transplantation surgery, which makes it possible to create the mosaic man.

In principle, a research project with human subjects is the consequence of the clinician’s dilemma and has its origins in his qualities of clinical observation and professional experience.

In this respect four ethical fundamental principles (8) are promoted, which give scientific value to a research project and at the same time respect the moral and physical integrity of human subjects: the principle of scientific interest and benefit of research, the principle of innocuity of research, respect for the person, and the principle of equity.

Surgical research implies high responsibility because, having as object of study the human being, it is as dangerous as it is useful. In this respect, one can find the Code of Ethical Principles for the surgeon-researcher, introduced by Resnik (quoted by 9) and which must be respected in practice, for the result of the work to have scientific value (table 2).

Table 1.

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<td>BENEFIT</td>
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<td>5.</td>
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The results of the research with scientific value will be communicated in speciality publications. Negative results with scientific value are accepted, which constitute a proof of respect for truth and stands as 'models to avoid for future research'.

Failure in research must not be seen as an impossibility to achieve the desired objective, but as an opportunity to reevaluate, to make new decisions, to discover new ways of research for the achievement of the desired task, and also must be written down as such for objectivity.

A very important part is played by the young doctor's training for research, which is dependent of their professional level, medical culture, and professional option.

In Roger D. E.'s opinion (9) 'medical school must have scientific education training in the field of the respectable discipline of research'.

This way, the researcher - surgeon, trained since medical school for research will fulfill his professional tasks.

I do not share the opinions according to which 'research is luxury or necessity', considering research a priority, and it is imperious for it to be a priority in contemporary medicine.

Recently, the researcher's activity was given a legal environment (411 / 2002), research being considered 'national priority' by the Law regarding the status of personnel working in research - development, emitted by the Government of Romania (10), in which the part, competences, responsibilities, rights and obligations of researchers are mentioned.

- Access to information sources
- Participation to scientific activities
- The right to publish books

Table 2

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<tr>
<th>Ethical principles</th>
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<tr>
<td>Honesty</td>
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<td>Accessibility</td>
<td>Caution</td>
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<td>Veridicity</td>
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<td>Efficiency</td>
<td>Freedom</td>
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<tr>
<td>Mutual respect</td>
<td>Legality</td>
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- The right to be supported by the working unit for professional training

Where are romanian and world research heading to?

One answer could be: high professionalism, interdisciplinarity, professional upgrade, thus being able to achieve main objectives such as: genetic therapy new techniques of organ transplantation, multiorgan transplantation, xenotransplantation, microsurgery, surgical robots and telemedicine, all this defining the concept of third millennium medicine.

In conclusion, Romanian surgical research has an undeniable part being in close contact with medical practice, which represent the source of research models and beneficiary of its results, however it is compulsory to respect ethical and deontological principles which give it scientific value.

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'SMALL-FOR-SIZE" SYNDROME IN LIVING DONOR LIVER TRANSPLANTATION

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Abstract

Small-for-size syndrome (SFSS) is a clinical syndrome which appears more frequently in cirrhotic patients following liver transplantation or extended hepatectomy. Persistent portal hypertension and portal over perfusion are considered the main features of the pathogenic mechanism; therefore the surgical approach should be adapted for resonating with portal inflow modulation, thus becoming the key element in preventing this syndrome. The most frequently used surgical methods for preventing small-for-size syndrome are: splenic artery ligation, splenectomy and portocaval shunt. The portocaval shunt is considered as a last resort, since diverting the blood from the portal vein could also have deleterious effect upon the liver and the portal flow. Herein below it is presented the case of a 38-year-old patient with HCV cirrhosis undertaking a liver transplantation (living-related donor, with right lobe). To correct hemodynamic changes occurring post reperfusion splenic artery ligation (SAL) has been performed, but this proved to be insufficient. Therefore, a porto-caval shunt was done in order to prevent small-for-size syndrome and to restore the flow in the hepatic artery, with good immediate result. However, in the postoperative period, the shunt had to be reversed because of the very low flow in the portal vein. After shunt ligation, the flow became normal in both portal vein and hepatic artery and the small-for-size syndrome did not develop. He was discharged in good condition, but did not come for the regular check-up. One year later he had to be retransplanted for portal vein thrombosis and liver failure.

Key words: "small-for-size" syndrome, liver transplantation

Introduction

Living donor liver transplantation - LDLT was successfully performed for the first time in 1989, when segments II and III were used. The method has been extended to adults in Japan, 1994 and USA, 1997, when right lobe was transplanted, thus becoming a viable alternative to cadaver donor transplantation and partially solving shortage of organs.

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The most important benefits of LDLT consist in reducing waiting time (178 days as compared to 354 days in cadaver donor transplantation), in shortening the cold ischemia time (an average of one hour compared to 8-12 hours in cadaver donor transplantation) and in having higher chances for a better quality liver and increased compatibility. From amongst the disadvantages of LDLT, donor complications should be mentioned. Another cause that limits LDLT success is the utilization of smaller grafts as compared to entire liver transplantation from cadaver (1, 2).

Large-scale utilization of LDLT has allowed for acquiring an in-depths expertise on surgical techniques as well as with respect to postoperative complications and solutions thereof.

One of such complications is represented by SFSS, a clinical syndrome which is difficult to be diagnosed (3, 4, 5), because it has no specific symptomatology. The most frequent signs are prolonged jaundice, reduced biliary production, coagulation disorder, ascites, digestive hemorrhage and urinary dysfunction (4, 6). The histological modifications reflect increased intrasinusoidal pressure and their main features are represented by vacuolar inclusions at the level of hepatocytes cytoplasm (swollen mitochondrias), hepatic steatosis, submassive hemorrhagic necrosis, collapse of Diesse spaces with inhibited biliary secretion and cholestasis around central vein (7, 8).

The syndrome is associated with a high mortality due to graft deterioration, including primary non-function (9, 10) and/or septic complications.

The severity of the syndrome requires an early diagnosis (intra-operative stage), upon the occurrence of post-perfusion graft congestion and monitorization of portal vein pressure. The causes of the hepatic congestion are: the size of the graft (small-for-size graft - SFSG defined by a ratio between the weight of the graft and body weight less than 0.8 %), deficient hepatic venous inflow and excessive portal inflow.

The solutions employed can be surgical, aiming at increasing the outflow, decreasing the inflow and enlarging the hepatic mass and medical (artificial liver, portal infusion of prostaglandin E1 associated with protease inhibitors, vasodilators and anticoagulants) (11, 12).

The aim of this article is to present the case of a patient with HCV cirrhosis who was transplanted with the right lobe liver from a living donor and who was exposed to SFSS risk due to high level of portal hypertension.

After the venous and arterial reperfusion, the graft was congested while the flow at the level of hepatic artery measured with the flowmeter was severely below the minimum recommended value.

Initially a splenic and gastro-duodenal artery ligation was performed thus managing to increase the arterial flow but with no effect on the hepatic congestion, signaling persistence of the portal hypertension. Under such circumstances, performance of a porto-caval shunt was decided.

Thus, SAL and the porto-caval shunt have ensured an adequate flow in the hepatic artery and protected the hepatic graft from the harmful consequences of an excessive portal inflow immediately post-transplantation.

After the lapse of 10 hours as of the intervention - interval throughout which the hemodynamic adaptation at the port system level has occurred - the shunt has become not only useless but dangerous due to the steal phenomenon, and therefore its ligation became imperative.

This solution proved beneficial both for preventing SFSS and for saving the hepatic artery of a patient undertaking a right lobe transplantation and although no references have been found in the studied literature, we suggest that this approach may be successfully used in similar cases.

Case report

The patient aged 38, with a height of 174 cm and weighting 74 kg, diagnosed with HCV - Child-Pugh B8 cirrhosis, undertook a right lobe liver transplantation from a living donor.

The recipient liver weighted 1,720 g. The volume of donor’s right lobe weighted at the pre-transplant stage amounted to 974 cm³ (measured by CT scan), and the hepatic graft weighted 1,085 g. GRWR (graft recipient body weight ratio) was 1.45%, therefore higher than 0.8 %, which is the ratio falling under the definition of SFSG.

Breakdown of operation time length is as follows: total hepatectomy - 315 minutes, anhepatic phase - 60 minutes (by fully clamping of inferior vena cava), cold ischemia - 135 minutes and warm ischemia - 25 minute.

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Implant of the hepatic graft was performed through hepatico-caval anastomosis (vascular reconstruction- fig. 1) and porto-portal and arterial anastomosis (fig. 2). Biliary reconstruction was performed through a bilio-biliary anastomosis (between two segmental ducts of the graft and the common bile duct of the recipient; a T tube was placed inside the bile duct (fig. 2).

Initially the graft was reperfused by declamping the vena cava and portal vein and subsequently by declamping the hepatic artery. Reperfusion consequences were graft congestion (fig. 3), systemic hemodynamic changes (hyperdynamic circulation, cardiac inflow increasing by 4.3 ml/min), low flow at the level of hepatic artery (14.4 ml/min) and excessive portal inflow (2 L/min), as measured by flowmeter (fig. 4).

The initial approach was to ligate splenic artery and gastro-duodenal artery to ameliorate the inflow in hepatic artery; this was partially obtained (the flow in the artery increased at 57 ml/min), but no effect was noticed on the hepatic congestion. Due to negative effects of portal hypertension over the graft and hepatic arterial inflow within the period following transplantation (13) we were forced into finding another solution to this problem.

Clamping of the portal vein was performed (hepatic artery buffer response) (14), the correlation between arterial and portal inflows being thus demonstrated: hepatic artery inflow increased from 57 ml/min (before clamping the portal vein) to 128 ml/min (after clamping) and congestion of the liver decreased significantly.

It was decided that a calibrated porto-caval shunt (8 mm) be performed, by inserting a Gore-tex grefon between the portal vein trunk and vena cava (fig. 5). Pursuant to such procedure, the
Small-for-size syndrome in living donor liver transplantation

following were registered: decrease of hepatic congestion, decrease of the blood inflow through portal vein (1.5 L/min) and of the cardiac output (8.9 ml/min from 9.3 ml/min) as well as an improved inflow through hepatic artery (70 ml/min). Ultrasound Doppler examination continued to show a turbulent inflow at the level of intrahepatic portal branches, as well as at the level of porto-caval shunt (fig. 6).

Two hours after the shunt (at the end of the surgical operation), hemodynamic parameters registered the following values: portal inflow - 0.6 L/min, cardiac output - 7.7 ml/min and arterial inflow - 65 ml/min.

Four hours postoperatively however, an increase of cardiac output to 11.2 ml/min was registered, while 10 hours thereafter it became 12.7 ml/min. Under such circumstances an ultrasound Doppler examination was performed, indicating a normal circulation at the level of hepatic artery (resistance index - 0.86), but the absence of venous inflow in the intrahepatic system (fig. 7).

Emergency reoperation was decided. Intraoperatively the flowmeter indicated an inflow of 48 ml/min at the level of hepatic artery and 0.34 L/min at the level of portal vein. Portocaval shunt ligation was performed (fig. 8), leading to the increase of blood inflow both in the hepatic artery (72 ml/min) and in portal vein (1.34 L/min) and to the decrease of cardiac output (8.6 ml/min). Ultrasound Doppler indicated that circulation in the portal and hepatic veins was normal (fig. 9).

On the 4th p.o.d. evolution was marked by the occurrence of a biliary fistula with medium flow (200 ml/day) associated with a cholestasis syndrome, requiring the second surgical reintervention on the 8th day: the bilo-biliary anastomosis was transformed into a Roux-en-Y hepatico-jejunostomy. The patient was discharged at 21 days post-transplantation.

The patient was non-compliant without follow-up.

In February 2006 he was admitted in another hospital for liver failure secondary to portal vein thrombosis and was retransplanted with normal postoperative course.
Discussion

LDLT for adults is a difficult procedure, which lacks a standardized surgical approach and has a high rate of complications (biliary and vascular), which may lead to graft dysfunction. SFSS occurs mainly in relation with aged donors or donors having hepatic steatosis, with small for size grafts.

Figure 7. Normal circulation at the level of hepatic artery (left), but the absence of venous inflow in the intrahepatic system (right)

Figure 8. Porto-caval shunt ligation

Figure 9. Normal circulation in the portal and hepatic veins
(SFSG), with receptors in an advanced stage of liver disease, associated with a high level of portal hypertension, in case of an insufficient venous drainage or of prolonged warm ischemia time (7). SSFS is defined by prolonged jaundice, intractable ascites, coagulation dysfunctions and urinary dysfunction associated with a high rate of septic complications.

The attempt to define SFSS and associated pathogenic mechanisms (excessive portal perfusion for a liver of too small size with insufficient outflow) has led to finding surgical and medical solutions, reducing the complications rate of LDLT or split.

Surgical solutions described by literature vary on a case-by-case basis due to the fact that associated mechanisms are different.

Thus, when SFSS is associated with SFSG, it is aimed at enlarging hepatic mass while in the other cases the main goal is to optimize the blood flow through portal and hepatic veins.

Enlargement of the graft may be obtained by auxiliary transplantation, by using two hepatic fragments, by harvesting the left lobe with the caudate lobe (15) or harvesting the right lobe with middle hepatic vein (VHM) (16, 17).

The auxiliary transplantation, which represented a therapeutical alternative before the 'right lobe era' is cited in the literature as an exceptional solution for SFSS, due to the fact that the surgical technique is very sophisticated (hepatectomy of a pathological liver can be very difficult), serial surgical interventions being needed as it is associated with a higher complication rate compared with LDLT ('steal' of portal blood between the native liver and the graft, biliary complications which may require retransplantation, acute cell rejection, transmission of the illness from the native liver to the graft) (4, 18, 19).

The utilization of two liver grafts poses, besides the disadvantage of a complicated procedure, the major impediment that it requires multiple donors (4), ensuring however an optimum hepatic volume for the receptor and maximum safety for the donors.

Utilization of right lobe in LDLT represented an important progress in hepatic transplantation from adult to adult. Technical complexity (linked with the necessity of an adequate venous drainage and with anatomical vascular and biliary variations requiring laborious reconstruction), as well as the key issue of ensuring maximum safety for the donor (reduced morbidity and zero mortality) are arguments pleading in favor of left lobe usage.

Such advantages of left lobe graft have been proved by certain studies undertaken by Asian teams (1, 20, 21), but which have not been confirmed by the transplantation clinics in western countries. Thus, European Liver Transplantation Register (ELTR) indicates a survival rate after passage of 1 year of 41% for the left lobe as compared to 73% for the right lobe, without mentioning whether modulation of portal inflow has been performed (22).

Utilization of right lobe with MHV has as advantage not only the enlargement of hepatic mass but also the optimization of outflow, ensuring an efficient venous drainage of anterior median sector (segments V-VIII). The technique is controversial as it increases, at least in theory, the morbidity of the donor. Certain studies indicate that there are no major differences between the two types of hepatectomies (right lobe without MHV and right lobe with MHV), if comparing blood loss and transfusion requirement, maximum level of liver function tests, the time needed for their normalization, complications and duration of hospitalization. Graft survival rate in case of right lobe with MHV is superior to the one registered for right lobe without MHV (86.2% and respectively 74.8%) (16, 23).

Transplantation of left lobe with caudate lobe permits an increase of hepatic volume by approximately 25%, being associated however with a decrease of blood losses for the donor as compared to prelevation of left lobe without caudate lobe (24).

Monitorization of outflow is performed through ultrasound Doppler. The parameters that proved useful in evaluating, to the most accurate degree possible, of an optimum inflow at the level of hepatic veins are represented by blood volume through hepatic veins (HVFV - hepatic vein flow volume) and the ratio between the pre- and post-transplantation HVFV. It is advisable that such ratio be higher than 80% as below such level the risk of complications (SSFS) increases significantly (25). Increase of outflow is obtained through a large anastomosis (minimum 40-45 mm diameter) (26) between hepatic veins and inferior cava vein (using triangulation technique or venoplasty), reconstruction of middle hepatic
vein (16, 17, 24, 25), reconstruction of the veins for segments V and VIII (17, 26) (fig.10) and of accessory veins (inferior right hepatic vein) with a diameter larger than 5 mm (24, 27, 28).

Due to the anatomical variety of the hepatic venous system (47% of potential donors who were checked had accessory hepatic veins and 22 out of those who donated the right lobe have necessitated implantation of the accessory veins in inferior vena cava, 17% of the patients presented a drainage vein of segment V in middle hepatic vein and also 17% had the VIII segment drained in the same vein, while in 23 of such cases anastomosis of middle hepatic vein with the vena cava was needed) (29). Reconstruction of outflow requires a large range of creative solutions, venous grafts being quite often needed (harvested from the recipient, portal vein - Y to I venoplasty, saphenous vein, inferior mesenteric vein, ovarian vein or using veins from a tissue bank) (30, 31, 32).

The third method for preventing SSFS consists in modulation of portal inflow (GIM - graft inflow modulation).

Monitorization of portal vein pressure (PVP) through a catheter placed in the inferior mesenteric vein in the anhepatic phase and in the first 14 days post-transplantation has shown that the increased values of PVP (>20 mmHg) noticed in the first 4 days are associated with an increased incidence of bacteremia, cholestasis, refractory ascites, increased prothrombin time and, finally, to a decreased chance of survival compared to patients with PVP < 20 mmHg. GIM, which determines an improvement of the inflow in the hepatic vein, permits prevention of such complications which characterize SFSS (13, 33).

The decrease of the inflow is obtained through SAL and/or splenectomy (9, 33, 34, 35, 36) and through various types of porto-systemic shunt.

SAL is a simple method, which reduces immediately the pressure in the portal vein from 10-20 mm Hg (average, 16 mm Hg) to 9-13 mm Hg (average, 11 mm Hg; P = 0,02)(30). The benefic hemodynamic effects of SAL, represented by the decrease of portal inflow and the increase of arterial inflow as measured by the Doppler, lead to significant improvement of clinical parameters: disappearance of refractory ascites, improvement of hepatic function, prevention of hypersplenism side-effects and finally, an increased survival rate (3,7). The negative consequences of ale SAL are reduced and rather rare: heart attack and/or splenic abscess, which may need splenectomy; pancreatitis or pancreas fistula, when the dissection of splenic artery results in trauma of the pancreas, such being prevented or treated by administration of octreotid.

Splenectomy insures a significant decrease of portal inflow; however the two risk factors related to infection have to be considered: immunosuppression and absence of the spleen. There are conflicting opinions in the literature with respect to the existence of an increased risk of infection for patients undertaking splenectomy. (34). Another severe complication that may occur post-splenectomy is portal vein thrombosis.

These surgical measures, SAL and splenectomy, do not always solve the problem of SSFS. If the inflow in the portal vein in post-transplantation stage is 3-4 times higher than the one measured at the level of receptor’s portal ram, SAL would not be enough, the porto-systemic shunt being required (7). Another reference for the necessity of a porto-systemic shunt is being represented by the value of the blood flow at the level of the portal vein as measured by flow meter (PVF). If PVF>260 ml/min/100 g, there exists the risk of SFSS occurrence. SAL is recommended when PVF<500 ml/min/g, while in the event PVF does not
exceed such level, SAL proves insufficient and performance of a porto-systemic shunt is required (3).

Utilization of a hemiporto-caval shunt (between a portal branch and the inferior vena cava) (fig. 11) determines a significant and persistent reduction of portal inflow with more than 50% compared to the initial value. However, the partial deviation of the portal inflow through the shunt poses a risk of hepatofugal inflow, which may lead to graft dysfunction, hepatic atrophy and portal vein thrombosis. Moreover, the shunt does not impede on the process of graft regeneration (in theory, the decrease of portal inflow could determine the slow down of hepatic regeneration) (4, 7, 36).

These observations support the idea that checking hemodynamic details (flowmetry) at the preoperative stage is important to determine whether the shunt is necessary (4, 7, 36).

Other porto-systemic shunts described in the literature as solving the SFSS issue are porto-caval shunt with graft interposition (37, 38) (fig. 12), porto-caval shunt with graft interposition between the portal branch and a hepatic vein (39) (fig. 13), mesocaval shunt with ligation of superior mesenteric vein beneath the shunt (40, 41) (fig. 14), portal vein-wrapping associated with plasmapheresis (42).

With reference to our case, it should be mentioned that SAL did not determine the decrease
of hepatic congestion and of the portal inflow, forcing us to find another solution. The calibrated porto-caval shunt, with the insertion of a Gore-tex graft with a diameter of 8 mm, determined the dissolution of the resistant ascites and to the significant decrease of portal inflow and amelioration of the arterial circulation. By combining the two surgical techniques, the graft has not been exposed to an excessive portal inflow in the first hour post-transplantation, thus efficiently preventing SFSS.

However, after the lapse of 20 hours, the shunt became dangerous due to almost fully deviation of the portal inflow through the shunt, requiring ligation. Eventually, the patient remained with the SAL, avoiding splenectomy and its consequences which are sometimes ill-fated due to immunosuppression, and without a porto-caval shunt which in time could have led to graft deterioration (due to insufficient portal inflow), encephalopathy, portal vein thrombosis and shunt thrombosis.

It is worth noting how quick the liver adjusted to the new circulatory regimen, since, after ligation of the shunt, the specific changes for small-for-size syndrome were no more registered.

This combination between SAL and temporary porto-caval shunt has not been found in the literature and may represent an efficient solution to SFSS.

The disadvantage of a reoperation for undoing the shunt seems irrelevant as compared to the benefits.

Conclusions

Porto-caval shunt was more efficient that splenic artery ligation for the adaptation of the liver at the new hemodynamic parameters, requiring close monitorisation.

Combined with splenic artery ligation, the porto-caval shunt induced the development of a poor flow state in the portal vein. In this event, reoperation with ligation of the shunt and reestablishment of an adequate inflow in both portal vein and hepatic artery is recommended.

Although that needed the ligation of the shunt 20 hours later, the final hemodynamic pattern allowed the survival of the liver.

The portal vein thrombosis can be explained by the presence of Gore-Tex graft (just ligated).

References

"Small-for-size" syndrome in living donor liver transplantation


SECONDARY CANCERS IN THE COURSE OF CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FLUDARABINE AND RITUXIMAB

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Abstract Major advances have occurred in our understanding of the biology, immunology and opportunities for treatment of chronic lymphocytic leukemia (CLL) in the last decade. It is known that CLL is characterized by progressive defects in humoral- and cell-mediated immunity. Quantitative and functional T-cell defects, NK and B lymphocyte dysfunction, low gammaglobulins and defective antibody-dependent cellular cytotoxicity (ADCC) have all been described in the setting of CLL. The etiology of such abnormalities is not entirely clear. In concert with each other, they account for the increased susceptibility of the CLL patients to major bacterial offenders and, possibly, secondary malignancies. Several surveys have defined CLL patients as a high-risk patient population for developing second neoplasms. Treatment of CLL further exacerbates immunosuppression by depleting both T and B immune effectors, thus facilitating infection by a large cohort of pathogenic organisms. There are data suggesting that second malignancies might also be more frequent and more aggressive after nucleoside analog therapy of CLL.1-4 This aspect has to be taken into consideration when treating patients with any of these potent drugs. We describe a patient who developed two secondary cancers in the course of his successfully treated CLL, initially with fludarabine and subsequently with rituximab in escalating doses. It is very important to recognize a second cancer early in patients with CLL.

Key words: chronic lymphocytic leukemia, secondary malignancies, immunosuppression, fludarabine, rituximab

Case Report

A 56-year-old white male, former heavy smoker, first presented in 1998 with night sweats and generalized lymphadenopathy. His peripheral WBC count was 80,000, mostly consisting of small lymphocytes. He had no anemia or thrombocytopenia. His lactic dehydrogenase and beta-2-microglobulin levels were normal. The periferal lymphocytes were CD5+, CD19+, CD20 weakly positive and CD23+. Surface immunoglobulin was demonstrable with monoclonality for lambda light chains. CD10 was negative. The diagnosis of chronic lymphocytic leukemia (CLL), intermediate risk by modified Rai staging system, was made. The patient was treated with six cycles of intravenous fludarabine (Fludara), with resolution of symptoms, followed by decrease in the size of lymph nodes and peripheral white blood cell count. His short-term side effects were grade 3...
anemia and thrombocytopenia, and he required transfusions on a few occasions. No hemolysis was documented in the same interval.

The course of his disease was uneventful until October 1999, when he was hospitalized for an episode of pneumococcal pneumonia. His condition improved with intravenous antibiotics, but all his immunoglobulin levels were significantly decreased. However, his CD4 and CD8 T-lymphocyte counts, as well as CD4/CD8 ratio were normal.

In September 2000, he developed an ulcerated lesion on his left anterior chest that was bleeding intermittently. The lesion, found to represent basal cell carcinoma (BCC) with typical morphologic features, was removed in October of the same year by the means of Mohs micrographic surgery. The BCC has not recurred to date.

In December 2000, the patient was started on weekly rituximab (Rituxan, Genentech/Idec) in escalating doses, up to 2,250 mg/m², as induction therapy, followed by the highest escalated dose monthly, as maintenance therapy. He tolerated the therapy well, with no infusion reactions or significant cytopenias, achieving a partial remission of CLL by June 2001.

He did well until December 2004, when he developed persistent cough with recurrent hemoptysis. Chest x-ray showed a thin-walled right upper lobe lesion. Subsequent computed tomography (CT) scan confirmed a cavitating lung mass in the upper lobe of his right lung (Fig. 1A) and showed extensive lymphadenopathy, unchanged since the previous examination and likely related to his CLL. Bronchoscopic biopsy was consistent with invasive, poorly differentiated non-small cell lung carcinoma (NSCLC). Further morphologic and immunohistochemical analysis favored squamous cell type (Fig. 1B). The tumor was EGFR positive/3+, HER-2/neu negative. A positron emission tomography (PET) scan was not done because it was felt not to be interpretable due to the CLL. Right upper lobectomy with mediastinal lymph node dissection was pursued instead. Pathology revealed a 2.7-cm squamous cell carcinoma with all bronchial, vascular and pleural margins free of tumor invasion. The removed peribronchial and mediastinal nodes were also negative for carcinoma, but infiltrated with small lymphocytes that were consistent with CLL. The final lung cancer staging was therefore IA (T1N0M0). The postoperative course was uneventful and no further chemotherapy was employed.

In April 2005, the enlarging lymph nodes, increasing WBC count and LDH level prompted a bone marrow examination. Bone marrow biopsy showed 40% cellularity and a focal and interstitial small lymphocytic infiltrate (Fig. 1C). The immunophenotyping was similar to that at diagnosis, except the cells were negative for CD20. Cytogentic revealed a normal male karyotype with no observed abnormalities. His serum IgG value was 365 mg/dl, IgA was 25 mg/dl and IgM was only 5 mg/dl. In the following two months, the patient received two courses of intravenous fludarabine, followed by reinstitution of rituximab. Shortly thereafter, he again developed hemoptysis and, at that time, CT scan showed a 3.5-cm right suprahilar mass that was re-biopsied. Pathology confirmed the original diagnosis of squamous cell carcinoma and platinum-based chemotherapy was commenced.

**Discussion**

Major advances have occurred in our understanding of the biology, immunology and opportunities for treatment of chronic lymphocytic leukemia (CLL) in the last decade. It is known that CLL is characterized by progressive defects in humoral- and cell-mediated immunity. Quantitative and functional T-cell defects, NK and B lymphocyte dysfunction, low gammaglobulins and defective antibody-dependent cellular cytotoxicity (ADCC) have all been described in the setting of CLL. The etiology of such abnormalities is not entirely clear. In concert with each other, they account for the increased susceptibility of the CLL patients to major bacterial offenders and, possibly, secondary malignancies.

Several surveys have defined CLL patients as a high-risk patient population for developing second neoplasms. Treatment of CLL further exacerbates immunosuppression by depleting both T and B immune effectors, thus facilitating infection by a large cohort of pathogenic organisms. There are data suggesting that second malignancies might also be more frequent and more aggressive after nucleoside analog therapy of CLL. (1-4) This aspect has to be taken into consideration when treating patients.
Secondary cancers in the course of chronic lymphocytic leukemia treated with Fludarabine and Rituximab

We describe a patient who developed two secondary cancers in the course of his successfully treated CLL, initially with fludarabine and subsequently with rituximab in escalating doses. It is very important to recognize a second cancer early in patients with CLL.

Although the indications to initiate treatment in patients with CLL remained basically the same over the last decade, the treatment of the disease has changed radically. The purine analogues fludarabine and cladribine have been demonstrated to be the most active drugs in CLL. Emerging as a new and effective combination, fludarabine, followed by rituximab, was administered in the presented patient.(6) Among the side effects of purine analogues, immunosuppression is an important one, resulting in prolonged decreases in CD4 lymphocytes and mandating prophylaxis of opportunists for a period of a few months. Although the short-term mortality in treated CLL is currently very low, long-term disease- and treatment-related complications are increasingly observed with improved overall

Figure 1A. Computed tomography scan showing the patient’s cavitating lesion in the upper lobe of his right lung.

Figure 1B. Low-power photomicrograph of lung tissue biopsy showing invasive squamous cell carcinoma. The tumor was HER2/neu negative.

Figure 1C. High-magnification of bone marrow biopsy specimen showing focal and interstitial small lymphocytic infiltrate. The well-differentiated lymphocytes were CD20 negative when tested.
survival. Long before the purine analogues became available, Greene et al pointed out in a large retrospective study the significantly elevated risk in CLL patients for malignant melanoma, soft-tissue sarcomas, and lung cancer. (7) A large European study by Mellemgaard et al found increased risks for cancer of the lung and prostate in men (RR=2.0 and 1.5 respectively) as well as even higher risks (RR=3 or higher) for non-melanoma skin cancer, renal cell carcinoma, and sarcomas in both sexes. They concluded that the risk is significantly increased for a number of cancer sites in persons with CLL. (8) In another study, Suzuki et al found secondary malignancies to be the major cause of death in 16% of evaluated CLL cases. (9)

Two or even three malignancies in one CLL patient were rarely seen in the past, but more such cases have been reported lately. One of the frequently encountered secondary cancers is a skin cancer. Malignant melanoma, Merkel-cell tumor, basal and squamous cell carcinoma of the skin (SCC) have all been reported in excess in the setting of CLL. Cohen et al have shown the development and quick dissemination of Merkel-cell carcinoma soon after receiving chemotherapy with fludarabine and rituximab for relapsing small lymphocytic lymphoma. (10) Larsen et al reported a case of SCC in a patient with CLL that relapsed locally after excision and metastasized to multiple distant sites, while on treatment with fludarabine. (11)

BCC in our patient presented early in the course of his CLL, yet after the use of fludarabine. It had typical clinical and histologic features and its course has been uncomplicated. The data in the reviewed literature showed increased clinico-histologic atypia of BCC in CLL (12) as well as significantly increased recurrence rates after Mohs surgery. (13)

An increased incidence of lung cancer of all major histologies has been observed in CLL. A large study by Parekh et al has shown that approximately 2% patients with CLL develop lung carcinomas. (14) In that study, however, 85% of the patients were smokers and lung carcinoma developed approximately a decade after the diagnosis of CLL. Another conclusion was that CLL and poor performance status limit treatment, particularly for patients with unresectable lung carcinoma. Patients who develop both diseases eventually die of lung carcinoma and not CLL or other solid tumors. (4, 14) Potti et al showed that HER-2/neu overexpression may be involved in the development/progression of lung cancer in patients with CLL and have an associated worse outcome. (15) Robak et al have shown the increased incidence of lung cancer in a cohort of patients treated with the purine analogue cladribine. (1) A significantly increased number of non-melanotic skin cancers were recorded in the same group.

The pathogenesis of hypogammaglobulinemia in CLL is poorly understood. It occurs in more than 50% of patients with CLL. At diagnosis, it may be noted in about 8% of patients, but its incidence increases significantly (up to 65%) with disease progression. Usually, all three immunoglobulin classes (IgG, IgA and IgM) are decreased, but in some patients only one or two may be low. Often, the IgM and IgA levels are decreased to a greater extent than the IgG level. With decreased immunoglobulin levels, one would also expect a defective ADCC as well. Significant hypogammaglobulinemia in the presented patient, along with other immune defects, may have resulted in increased susceptibility not only to pneumococcal infection, but perhaps to secondary tumors as well.

Used in the treatment of a large range of B-cell malignancies, the monoclonal antibody rituximab is an active agent in CLL, especially if utilized in escalating doses. (5, 16) The drug targets the CD20 antigen present on the surface of neoplastic and normal B lymphocytes. It is thought to cause apoptosis and cell lysis via ADCC. According to a study conducted at our cancer center, the agent induced a good level of response in CLL patients, with the overall response rate of 88%. Interestingly, our patient’s malignant B-cells in the bone marrow, done in April 2005, did not express CD20 antigen, as an evidence of vigorous treatment with rituximab.

Our experience with this agent points out that escalating doses of rituximab are effective in treatment of non-bulky disease. However, high doses of rituximab might have also depleted the normal B-cell clones and impaired normal antibody response in the patient. Two studies have shown depletion of normal B-cells by a single standard dose of Rituximab that persisted for 6 to 9 months. (17, 18) Rituximab interfered with both elicited humoral response to the recall antigens as well as with the memory response when administered prior to antigen exposure. (18)
These phenomena might be even more dramatic for immune response in case of high-dose rituximab. This interference might have precluded the mounting of an efficient antitumoral response as well.

The patient presented in this case exemplifies many aspects of second cancers in treated CLL. His initial secondary tumor, BCC, presented early in the course of his CLL, yet after the use of fludarabine. His CLL was in continuing partial remission for a few years prior to his diagnosis of lung cancer. Our patient developed two of most commonly encountered secondary malignancies in CLL, and his more serious disease was initially localized. Furthermore, his lung cancer was HER-2/neu negative, yet extremely aggressive. In spite of the fact that surgical intervention rendered the patient disease-free, his lung cancer recurred after only 5 months. Unfortunately, despite long-term partial response to treatment of his CLL, he will most likely succumb to his extremely aggressive lung cancer.

Progressive intrinsic immune deficiencies in this CLL patient, particularly severely decreased immunoglobulin levels, may have played an important role in the development of his second tumors. Furthermore, additional immunosuppression caused by use of the purine analog fludarabine might have contributed to the occurrence of both cancers and the increased level of aggression of his lung malignancy. Moreover, rituximab in escalated doses may have interfered with the antibody response against the tumor antigens and the antigen-presenting function of normal B cells, perhaps further facilitating lung cancer offensive and growth.

The created situation of immunosuppression, along with the pattern of second cancers seen in CLL, is similar to the one seen in solid organ or autologous stem cell transplant (ASCT) recipients. (19) This resemblance, along with the presented patient’s situation, makes the case for heightened surveillance and regular follow-up of all CLL patients. In addition to the usual screening for breast, cervical, prostate and colon cancers, they need regular complete skin examinations. Seven steps to safer sunning such as avoiding the sun, using a sunscreen, wearing a hat, wearing sunglasses, covering up, avoiding artificial tanning, and checking skin regularly cannot be overemphasized. It is perhaps appropriate to consider early lung cancer screening in CLL patients, especially in those over the age of 50, with a history of heavy smoking and treatment with a purine analogue. More studies are necessary to determine the optimal screening and follow-up schedule for these patients in order to minimize the death toll of secondary cancers. Looking forward, improvements in selection of chemotherapeutic and/or immunotherapeutic regimens will hopefully reduce long-term complications in order to allow their curative potential to be exploited to the fullest.

We certify that any affiliations with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, honoraria, expert testimony) is disclosed above. No financial support was used for this work.

We certify sufficient participation of each author in the conception, design, analysis, interpretation, writing, revising, and approval of the manuscript.

We attest that no other article by the author substantially similar in content has been published or is currently being considered for publication.

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GUIDELINES FOR THE TREATMENT OF CROHN'S DISEASE IN ADULTS

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A. Inflammatory Crohn's disease

The classification of Crohn's disease according to severity can be made using Crohn's disease activity index (CDAI, a complex, numeric index frequently used in clinical trials) and/or American Gastroenterological Association (AGA) criteria, which are more clinical, easier to use.

Mild disease is defined by a CDAI between 150 and 220 and by AGA criteria as: ambulatory patient, which feeds normally, with a weight loss under 10% of body weight, no fever, no signs of dehydration, no abdominal mass or tenderness, also with no signs of intestinal obstruction.

Moderate disease is defined by a CDAI between 220 and 450. The patient presents with intermittent vomiting, palpable abdominal mass, but without signs of obstruction, with a weight loss greater than 10% of body weight, or the patient is not responsive to the medication for mild disease.

Severe disease is defined by a CDAI over 450, the patient being usually malnourished, or with evidence of complications as obstruction, abscesses, and with persistent symptoms despite intensive treatment.

The management regardless of the form or localization of Crohn's disease must begin with the recommendation that it is mandatory for all patients to stop smoking.

I. Treatment for ileocaecal Crohn's disease

1a. Mildly active form

In the treatment of mild Crohn's disease localized at distal ileum and/or right colon the preferred drug is Budesonide in dose of 9 mg daily because of its efficacy which was proven to be superior to both placebo and 5 ASA and also due to its favorable safety profile. Alternative approaches are: Mesalazine dosed at a minimum of 4g/day (lesser doses proved ineffective), which has a more limited benefit. Clinical studies showed that the reduction of CDAI in patients treated with Mesalazine as compared with those on placebo was only of 18 points (-63 vs. -45, p = 0.04), value that although ensures a statistically significant difference, from a clinical point of view the magnitude of symptom reduction is not so impressive. In the treatment of ileocaecal Crohn's disease antibiotics are not recommended. Also, nutritional therapy is not recommended in this (mild) form of disease.

1b. Moderately active form

As for mild form, Budesonide 9 mg is effective and should preferably be used. Alternatively, treatment with corticosteroids such as Prednisone in dose of 1mg/kg has similar efficacy but causes more side effects. Antibiotic use is recommended in association with corticosteroids in cases where septic complications are suspected. Nutritional

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therapy can be used in order to correct nutritional deficiencies, only as adjunctive therapy. Nor Infliximab treatment or surgical treatment is recommended in the moderate form of disease.

1c. Severely active form

Intravenous corticosteroids are indicated. Association with immunomodulators such as Azathioprine is recommended especially in patients who are not at the first flare of disease because Immunomodulators have corticosteroid sparing effect and also are effective as maintenance treatment. In case of Azathioprine intolerance Methotrexate can be used instead. Infliximab treatment is recommended for patients resistant, intolerant or with contraindications to corticosteroids. Unlike other disease locations, because of its good outcome, for ileocecal Crohn’s disease the indication for surgical treatment can be made earlier in the disease evolution. Surgical therapy is recommended in case of lack of response after 2-6 weeks of medical treatment. Antibiotics are reserved for septic complications of disease (abscesses, local tenderness, fever).

2. Treatment for colonic Crohn’s disease

2a. Mildly active form

Treatment with Sulfasalazine or Mesalazine orally in dose of at least 4 grams is efficient but has many side effects (especially Sufasalazine). Sulfasalazine is particularly indicated for patients with concomitant extra intestinal articular manifestations of disease. In association, as adjuvant or as sole therapy topical preparations of Mesalazine can be used for left sided disease.

2b. Moderately and severely active forms

Treatment with orally (for moderate disease) or intravenous (for severe disease) corticosteroids is recommended for colonic Crohn’s disease. Association with Azathioprine is recommended in patients who experienced previous flares of disease. Methotrexate can be administered in patients intolerant to Azathioprine. Infliximab treatment is recommended for Crohn’s disease refractory to or intolerant to corticosteroids. Surgical treatment is reserved for severe disease flares unresponsive to medical treatment. In the case of patients who are intolerant to corticosteroids and in whom Infliximab treatment is not possible, antibiotics can be used, such as Metronidazole in dose of 10-20mg/kg body weight. However, clinical studies showed that treatment with Metronidazole can induce response but not remission of disease. For that reason and due to its potential side effects Metronidazole could not be recommended as first line therapy. The treatment with Mesalazine is not recommended in severely active forms of colonic Crohn’s disease but could be used in moderate forms of disease either orally or as topical forms. Nutritional therapy has only an adjuvant role in patients with colonic Crohn’s disease, for the correction of malnutrition.

3. Treatment for ileal or jejunal Crohn’s disease

3a. Mildly active disease

For this form of disease the recommended treatment is with Mesalazine (a form with intestinal release, as Pentasa) or with antibiotics (Metronidazole or Ciprofloxacin). Also nutritional therapy as sole therapeutic modality could be considered for this location in mild forms.

3b. Moderately and severely active disease

Corticosteroids are recommended, and Budesonide is preferred (due to its safety profile) if the disease involves exclusively the terminal ileum. Because Crohn’s disease of the small bowel could be associated with more severe consequences compared with colonic disease (malnutrition, repeated surgical interventions resulting in short bowel syndrome), it is recommended that immunomodulator therapy (Azathioprine) should be started early in the disease evolution, at first flare of the disease, especially in extensive cases, when over 100cm of small bowel is involved in the disease process. In patients intolerant to Azathioprine, Methotrexate can be used. Infliximab use is recommended in cases resistant to corticosteroids. Surgical treatment is reserved for obstructive, old, fibrous strictures. Healing of inflammatory strictures should be tried with medical treatment first and in general surgical treatment should be as conservative as possible. Resections should be limited at minimum in order to avoid short bowel syndrome, the recommended surgical approach being stricturoplasty. As stated early, patients with small bowel Crohn’s disease are frequently malnourished, so adjunctive nutritional therapy
has an important role in the management of those patients.

4. Treatment for esophageal or gastroduodenal Crohn’s disease

For this location the recommended treatment is with corticosteroids in association with proton pump inhibitors. Early in the disease evolution is recommended the introduction of an immunomodulator (Azathioprine or in case of intolerance, Methotrexate). For cases which are refractory to corticosteroid therapy, Infliximab is indicated. In obstructive cases surgical treatment or endoscopic dilatation procedures are indicated.

5. Treatment of early relapse, corticosteroid dependent or corticosteroid refractory Crohn’s disease

In cases of an early Crohn’s disease relapse (defined as a flare of disease occurring less than three months after a previous flare) initiation of immunomodulator treatment is recommended (Azathioprine or Methotrexate). Also, immunomodulator treatment is recommended for corticosteroid dependent (relapse of disease at the intent of corticosteroid dose reduction) and corticosteroid resistant (lack of response to corticosteroids) Crohn’s disease. In cases of immunomodulator failure, Infliximab treatment can be used. If no response is obtained after Infliximab administration, surgical treatment should be considered.

6. Treatment for the maintenance of medically induced remission

Regardless of the severity of Crohn’s disease flare, the maintenance treatment should be started at the same time as the treatment for the induction of remission.

With the exception of corticosteroids, which proved to be ineffective in the maintenance of remission for Crohn’s disease, all the rest of the therapeutic agents used for induction of remission can be used also for the maintenance of remission. A special note should be made regarding Budesonide: its use can delay the occurrence of a new disease flares but only during the first year of Budesonide use, because after this time period it was proved that the rate of relapses is similar for Budesonide to that for placebo.

The role of Mesalazine in the maintenance of remission in Crohn’s disease is limited. So, if Mesalazine use proved to be modestly efficient in ileal Crohn’s disease, for the colonic location the results are not very convincing. However, especially if remission was obtained using Mesalazine, the maintenance treatment with Mesalazine could be tried in colonic Crohn’s disease, although there is not consistent evidence for the efficacy of this approach. It is recommended that the daily dose of Mesalazine used as maintenance treatment should be greater than 2 grams. The therapy should be maintained long term, generally 3-5 years (at least two years of complete remission). Unlike ulcerative colitis, Mesalazine use did not proved to have a role in the prevention of colorectal cancer in colonic Crohn’s disease. Regarding Sulfasalazine and its efficacy in the maintenance of remission, there are not sufficient data in the literature to draw a conclusion. Also, due to its numerous side effects it cannot be routinely recommended.

The role of antibiotics in the maintenance treatment of Crohn’s disease is not yet established and their potentially side effects especially after long term use (for Metronidazole) make the use of antibiotics of limited utility.

Azathioprine is efficient and is the main therapy indicated for the maintenance of remission especially when the remission was induced with corticosteroids. Azathioprine is also recommended in patients who experienced more than one flare of disease during one year. For patients already in maintenance treatment with Azathioprine in case of new disease flares the recommended approach is to augment the Azathioprine dose. Also, switching from Azathioprine to Methotrexate could be tried. The Azathioprine treatment should be continued for at least four years of complete remission, although even after six years a benefit persist for the patients on therapy as compared with patients in whom treatment was interrupted. In case of Azathioprine intolerance, Methotrexate is recommended.

Infliximab as maintenance treatment, administered at regular intervals, every eight weeks, is efficient and is recommended in cases of intolerance or resistance to the other therapeutic means.
7. Surgical treatment has the following indications in Crohn's disease:

a. For ileocaecal, localized Crohn's disease with obstructive symptoms surgical treatment is indicated to obtain remission, and is recommended early in the evolution of the disease since this patients will sooner or later need surgical therapy and the results of surgical treatment are very good (half of patients operated on will not need a second surgical intervention).

b. Small bowel Crohn's disease with associated abscess: recommended treatment is surgical or percutaneous drainage, in association with antibiotics.

c. Small bowel Crohn's disease with obstructive symptoms: stricturoplasty is recommended.

d. Colonic Crohn's disease resistant to medical treatment has surgical indication. The recommended intervention is limited resection, with the removal only of the macroscopically diseased colonic part. Two segments of the colon can be resected if the disease affects only the proximal and the distal ends of the colon. Colonic stricturoplasty is not recommended. In cases of short length strictures endoscopic dilatation can be tried. Also, in cases of total proctocolectomy, an ileal pouch is not recommended.

8. Treatment for the maintenance of surgically induced remission

Mesalazine in high doses (at least 2 grams/day) is efficient and recommended for the prophylaxis of recurrence after surgically induced remission. The treatment should be maintained for at least two years postoperatively. Metronidazole administered for three months after ileocecal resection proved to be effective in the prevention of relapse but its efficacy is maintained only in the first year following surgery. Azathioprine is effective for decreasing postoperative recurrence and its use should be considered especially in complex forms of Crohn's disease, in patients with an elevated risk for recurrence (with extensive forms, over 100 cm of small intestine involved, with active smoking, or with colonic disease) or in which recurrence of disease would have deleterious effects. Azathioprine is indicated in case of severe endoscopic lesions diagnosed postoperatively even if the patient is asymptomatic.

B. Fistulizing Crohn's disease

Classification of perianal disease in simple or complex is based on anatomical criteria as used by Parks (Fig. 1): simple fistulas are low superficial (A), inter-sphincteric (B) and trans-sphincteric (C) while complex fistulas are high trans-sphincteric, supra-sphincteric (D), extra-sphincteric (E), fistulas with internal opening above the dentate line or with multiple external openings.

1. Treatment of simple perianal fistula

Surgical treatment is recommended: noncutting seton placement or fistulotomy in association with antibiotics (Metronidazole or Ciprofloxacin)

2. Treatment of complex perianal fistula

As first choice therapy the association antibiotics and/or immunomodulators and surgical treatment (seton placement) is recommended. If an abscess is present it should be surgically drained. Another choice is Infliximab, which is also effective for the treatment of perianal fistulas. Intravenous Cyclosporine can be used but for its efficacy to persist the continuous administration of Cyclosporine in oral form is needed, because after discontinuation of therapy relapse is the rule. The numerous adverse events associated with long term Cyclosporine treatment make the use of this medication reserved for cases in which the other therapies...
failed. The administration of Tacrolimus is associated with a reduction in the severity of symptoms but not with remission of the disease and is also not currently recommended, being reserved for resistant cases.

3. Maintenance of fistula closure

Azathioprine treatment is recommended, in association or not with long term seton placement. For patients in whom Azathioprine is not effective, maintenance treatment with Infliximab is indicated, and it must be continued for at least one year. Methotrexate treatment is an alternative in patients intolerant or resistant to Azathioprine or Infliximab.

4. For enterovescical fistulas surgical treatment is indicated

5. For enterocutaneous fistulas developed after surgical intervention the treatment recommended is surgical reintervention after correction of patient’s nutritional status. In case of spontaneous fistulas medical treatment is recommended using the same therapeutic means as for perianal fistulas. Alternatively, surgical treatment with concomitant resection of the diseased intestine can be an option.

Selective bibliography

QUALITY ASSESSMENT AND CONTROL IN THE RESEARCH MICROBIOLOGY LABORATORY

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Abstract

Quality Assessment in Microbiology Laboratory attempts to identify, monitor, evaluate, and improve practices related to patient care. Quality Control (QC) is a concept that includes evaluating product performance, comparing this performance to stated goals and taking action when the product fails. In the microbiology laboratory the accuracy of the test information is dependent upon the specimen quality; the correlation of the test method with clinical data; the performance of the test procedures personnel, media, reagents and instruments; and the method for the reporting results. Microbiology QC programs should address the following elements: test methods and procedures, verification and validation of tests, content of procedure manuals, content and storage of records and reports, competency of personnel, proficiency testing and laboratory error. Essential Criteria for Quality Systems of Medical Laboratories have been published recently by the European Community Confederation of Clinical Chemistry (EC4) Working Group on Harmonization of Quality Systems and Accreditation. The Essential Criteria address the majority of critical aspects of quality management in the medical laboratory - microbiology in particularly in this study.

Key words: quality control, research microbiology laboratory, quality assessment

Introduction

The QC program is designed to continuously monitor these elements, identify problems in test performance or process and correct the problems.

Quality Control is the process or system for monitoring the quality of laboratory testing, and the accuracy and precision of results.

Routinely collect and analyze data from every test run or procedure; Allows for immediate corrective action, establish written policies and procedures, corrective action procedures, train all staff, design forms, assure complete documentation and review.

Quality Control it is qualitative and quantitative. For implementation this QC need to: selection and managing control materials, analysis of QC data and monitoring quality control data. (Table 1)

The Control Qualitative test measures the amount of a substance present. The Qualitative test determines whether the substance being tested for is present or absent.

Quality control is performed for both, system is somewhat different.

Implementing a QC Program -Quantitative Tests: select high quality controls; collect at least 20 control values over a period of 20-30 days for each level of control; perform statistical analysis; develop Levey-Jennings chart; monitor control

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values using the Levey-Jennings chart and/or Westgard rules and take immediate corrective action, if needed and record actions taken.

**Microbiology QC**

Microbiology QC programs should address the following elements: test methods and procedures, verification and validation of tests, content of procedure manuals, content and storage of records and reports, competency of personnel, proficiency testing and laboratory error.

QC is a concept that includes evaluating product performance, comparing this performance to stated goals, and taking action when the product fails.

In the microbiology laboratory the accuracy of the test information is dependent upon the specimen quality; the correlation of the test method with clinical data; the performance of test procedures, personnel, media, reagents, and instruments; and the method for the reporting of results.

In the microbiology laboratory the accuracy of the test information is dependent upon the specimen quality; the correlation of the test method with clinical data; the performance of test procedures, personnel, media, reagents, and instruments; and the method for the reporting of results.

Check the sterility, ability to support growth, selective or inhibitory characteristics of the medium, biochemical response.

Check for growth of fastidious organisms on media of choice - incubate at time and temp recommended.

Quality Control: Stains and Reagents. Gram stain QC - use gram positive and gram negative organisms to check stain daily; Check as used - positive and negative reactions

Evaluation of the Quality System with: internal audit (there should be an estimate of costs of the quality system, including costs of duplicates, control samples and also costs of overhead, and effects on costs of failures and corrective actions) and external audit (in clinical audit, services should be checked for clinical effectiveness and cost efficiency).

**Test method and Procedures**

A QC program monitors, evaluates and documents the performance of all aspects of a test procedure. This includes the quality of the specimen; the performance of reagents, media, and instruments; and the review of test results for error. Appropriately collected and transported specimens are essential for quality microbiology results. The laboratory should monitor the quality of specimens received and be proactive in improving performance. Reagents, supplies, and strains are labeled to indicate identity, concentration, storage requirements, preparation and expiration dates, and if applicable, the type of safety hazard associated with their use. In general, negative and positive controls are required for qualitative tests (e.g., catalase and oxidase test), and two controls with different titers or concentration are necessary for quantitative tests (e.g., serology).

The quality and medical relevance of the microbial test information are dependent upon the timeliness and accuracy of the reports. The laboratory should establish clinically useful turnaround times (TAT s) for critical tests such as body fluid smears, acid-fast bacillus smears, and cultures through consensus of those involved in patient management.

Selecting Control Materials. Calibrators

- Has a known concentration of the substance (analyte) being measured
- Used to adjust instrument, kit, test system in order to standardize the assay
- Sometimes called a standard, although usually not a true standard

Known concentration of the analyte, use 2 or three levels of controls and include with patient samples when performing a test, and used to validate reliability of the test system.

Important Characteristics: values cover medical decision points, similar to the test specimen (matrix), available in large quantity, stored in small
aliquots, ideally, should last for at least 1 year, often use biological material, consider biohazardous.

Sufficient material from same lot number or serum pool for one year's testing, may be frozen, freeze-dried, or chemically preserved, requires very accurate reconstitution if this step is necessary, always store as recommended by manufacturer.

Types of Control Materials: assayed (mean calculated by the manufacturer and must verify in the laboratory), unassayed (less expensive and must perform data analysis), 'Homemade' or 'In-house' (pooled sera collected in the laboratory, characterized and preserved in small quantities for daily use).

Establish a storage protocol: store at -20°C, in use vials stored at 4°C, use 0.5 ml vial maximum of one week, freeze-dried (requires accurate reconstitution), chemically preserved.

A certain amount of variability will naturally occur when a control is tested repeatedly.

Variability is affected by operator technique, environmental conditions, and the performance characteristics of the assay method. The goal is to differentiate between variability due to chance from that due to error. The degree of fluctuation in the measurements is indicative of the 'precision' of the assay. The closeness of measurements to the true value is indicative of the 'accuracy' of the assay.

Quality Control is used to monitor both the precision and the accuracy of the assay in order to provide reliable results.

Documentation is the cornerstone of a QC program; and all methods, policies, test results and corrective actions must be recorded (Table 2).

Verification and validation of tests. New test methods of high complexity must be verified for accuracy and precision before they are used for patient testing and the laboratory must validate existing methods. Validation of an existing verified test documents that the test continues to perform satisfactorily on the basis of QC data, proficiency testing and correction with clinical data.

Content of procedure manuals. The laboratory’s policy and procedure manual must contain all material relevant to the operation of the laboratory and production of patients’ test results. The procedure’s format where appropriate should generally adhere to the recommendation specified in CLSI/NCCLS publication GP2-A3.

Content and storage of records and reports. The laboratory must maintain sufficient records to document all aspects of testing performed on a patient’s specimen. The test requisition should contain the patient’s name or identifier the date of collection of the specimen, the date that the specimen was received in the laboratory, the name of the test requester, and the test requested.

Stock QC organisms. Organisms to be maintained must be adequate to check all media and test systems:

- E. coli - MacConkey, EMB, susceptibility tests
- Staphylococcus aureus - Blood agar, Mannitol Salt, susceptibility tests
- Neisseria gonorrhoeae - chocolate agar, Martin-Lewis

Competency of Personnel. The competency of experienced microbiologist may be verified by review of work cards, interpretation of unknowns, the use of proficiency samples direct observation, or a written examination.

Proficiency Testing. All microbiology laboratories must participate in an external proficiency program approved by the U.S. Department of Health and Human Services that reflects the laboratory’s specialty and level of expertise. Proficiency testing is a tool than can be used to assess a laboratory’s performance compared to the laboratory’s CQI program.

Laboratory Errors. Most errors detected relate to preanalytical mix-ups and occasionally post-analytical reporting errors that are brought to the laboratory’s attention by a puzzled provider. In general analytical errors are unusual.

Detecting Errors. Many organisms have pre-
dictable antimicrobial test results, ex:
- Staphylococcus spp. are usually susceptible to vancomycin.
- Streptococcus pyogenes are always susceptible to penicillin.
- Klebsiella pneumoniae are resistant to ampicillin.

**Reference standards and reference materials
ISO 17025:1999**

Reference standard. The laboratory shall have a program's and procedure for the calibration of its reference standards. Such reference standards of measurement held by the laboratory shall be used for calibration only and for no other purpose, unless it can be shown that their performance as reference standards would not be invalidated.

Reference materials. Reference materials shall, where possible, be traceable to SI units of measurement, or to certified reference materials. Internal reference materials shall be checked as far as is technically and economically practicable.

Intermediate checks. Checks needed to maintain confidence in the calibration status of reference, primary, transfer or working standards and reference materials shall be carried out according to defined procedures and schedules. (Table 3)

Transport and storage. The laboratory shall have procedures for safe handling, transport, storage and use of reference standards and reference materials in order to prevent contamination or deterioration and in order to protect their integrity.

Additional procedures may be necessary when reference standards and reference materials are used outside the permanent laboratory for tests, calibrations or sampling.

If you encounter an unusual pattern, rule out error by checking identification of organisms and repeat antimicrobial susceptibility test. Report if repeat testing yields same result, or refer the isolate to a reference laboratory for confirmation.

**Conclusion**

The introduction of total quality systems in medical and clinical laboratories and accreditation of these laboratories is gaining more and more interest. In several countries laboratories have set up quality systems, and accreditation schemes are also operating. The standards of these schemes have much in common although several differences exist. There exists uncertainty in several countries on the choice of a system. Laboratory specialists are confronted with a new way of thinking concerning the management and daily practice of their laboratories.

Laboratory specialists are confronted with a new way of thinking concerning the management and daily practice of their laboratories. It is not clear, which standards should be used as a basis, and certainly not how to interpret such standards (3). Particularly in the European Union, harmonization of criteria for quality systems is desirable. The success of a CQ program rests upon the commitment of the organization's leadership to the concept and to providing the necessary resources to implement the program.

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The goal of this monographic work is to reflect the current standard of the modern liver surgery. Many impressive moments had been prominent in the last decades, such as the successful liver transplantation from cadaver donor in 1967, the advancement of the hepatic resections in the 90s and the different types of liver transplantation from living donor which gain their well deserved place in the new millennium. All those tremendous moments are not essential only for liver surgery, but also for the contemporary medicine.

In accomplishing this paperback we based mainly on the experience of the Fundeni Clinical Institute (FCI), where presently resides the largest series of hepatic resections and is implemented the national program of liver transplantation. FCI is the place where the first successful liver transplantation in Romania was performed - 15th of April 2000, followed by the first liver transplant from living donor, domino transplantation or split liver transplant, significant moments for Romanian medicine that placed our country on the international liver transplantation map.

This book is the result of the combined work of prominent specialists from FCI as well as other foreign experts that actively caught up in the national program of transplantation, such as Andrei Stieber (USA), Domenico Forti, Massimo Malago, Andrea Gasperi (Italy), Christoph Broelsch (Germany).

In order to achieve the final form of this thesis, we aimed to realize a compressed vision, which would allow the understanding of the liver
surgery considering the complexity of its anatomy, physiology and pathology.

The chapters about liver pathological issues had been elaborated in strict correlation with imagistic examples or data from modern interventional radiology techniques that brought significant changes in the diagnosis and treatment of liver diseases.

The tumoral liver pathology, either benign or malignant, reflects mainly the great experience of the FCI in this matter. The malignant tumors are referred to as from the multidisciplinary treatment point of view, including some innovative therapeutic techniques.

Thus, there are two chapters that refer to the hepatic resections, written by two authors with great experience, that bring out complementary points of view. Considering the rapid development of liver surgery and the fast progression of the new therapeutic methods, some chapters in this book might reflect more the authors' own experience than a synthesis of the information in the literature of specialty regarding this matter.

The chapters dedicated to the liver transplant lay emphasis on the importance of the 'national transplant program', including all its components - brain-dead donor, living donor, transplant management crew, surgery for the recipient. These chapters hold together the great experience of important authors from centers like Atlanta (Emory University), Essen (Clinical University Hospital), Milano (Niguarda Hospital).

Pediatric liver transplantation establishes the level that was achieved nowadays, especially from the point of view of novel techniques, particularly the living donor transplantation.

We also approached themes like xenotransplantation, presenting the actual standing of this matter, as well as the current perspectives on the transgenic species, which will be used in the future as donors.

General surgeons and particularly those interested in liver surgery will find this book useful because of its concise format and its breadth of information. Also hepatologists, immunologists, radiologists, anesthesiologists or any other specialists interested in hepatic diseases with surgical indication will find it attractive because of the information this book brings.

This thesis does not claim that exhausts the subject presented, as in the last years liver surgery has become an extremely complex field, in permanent development. That's why we are looking forward to in receipt of any comments or reviews from our readers, hoping these will be useful for a future edition of this book.
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