Eosinophilic GI Disorders (EGID) following Immunosuppression for Liver Transplantation

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Abstract

Background: Eosinophilic gastrointestinal disorders (EGID) are a group of inflammatory gastrointestinal disorders, characterised by inappropriate eosinophil infiltration and symptoms that affect one or more parts of the GI tract, in the absence of extra-intestinal causes. They include eosinophilic oesophagitis (EO), eosinophilic gastroenteritis (EG) and eosinophilic colitis (EC), all of which can occur following immunosuppression for liver transplantation.

Aim: To present a review of the recent literature on EGID following immunosuppression for liver transplantation in order to clarify their diagnosis and treatment.

Methods: We performed a PubMed search for EGID, eosinophilic oesophagitis (EO), eosinophilic gastroenteritis (EG) and eosinophilic colitis (EC), associated with liver transplantation, their clinical presentation, diagnosis and treatments.

Results: In the liver transplant population, prevalence of EGID is up to one hundred times greater than the non-transplant population, making it a significant contributor to post-transplant morbidity. EGID affects individuals of all ages, favouring those in the third and fourth decades, and males over females. There is a strong association between these conditions and calcineurin inhibitors, tacrolimus and CsA, with tacrolimus appearing to confer a higher risk for the development of eosinophilic disorders.

Diagnosis of EGID depends on endoscopic and histological features, due to similar non-specific symptomatology of EGID but distinct endoscopic and histological features.

The mainstay of treatment for EO, EG and EC is systemic steroid therapy, however some specific therapies have been suggested including biologics such as mepolizumab (anti-IL-5 monoclonal antibody) for EO, octreotide (somatostatin analogue) for EG and montelukast (LTD4 receptor antagonist) for all three conditions. Empirical dietary elimination may also provide symptomatic relief.

Conclusion: EGID is an important but under-recognised complication of immune suppression, particularly of the drugs that predominate current anti-rejection therapy for liver transplantation. They are quite common and likely to impact on the patient’s quality of life. In patients presenting with non-specific GI symptoms, there must be a high index of suspicion for EGID, prompting further investigation through upper and lower endoscopy and histological analysis.

Modification of the immunosuppressive regime can contribute to reducing risk of relapse and treating active or refractory episodes. Therefore, in patients suffering from EGID, management of their immune suppression becomes important in control of the condition, rendering EGID a key factor in the design of their tailored immunosuppression.

Keywords: Eosinophilic gastrointestinal disorders; Eosinophilic oesophagitis; Eosinophilic gastroenteritis; Eosinophilic colitis; Eosinophils; Liver transplant; Tailored immunosuppression

Introduction

Liver transplantation is universally accepted as the standard of care in those suffering from severe hepatological illness refractory to medicinal therapies, in particular end-stage acute or chronic liver disease. The success of liver transplantation is dependent on the use of immunosuppressant drugs, such as tacrolimus and cyclosporine A. The United Network for Organ Sharing (UNOS) has reported survival rates at 3 years and 10 years post-transplantation at 80% and 50% respectively [1]. Although effective in the prevention of acute cellular rejection, immunosuppression is often accompanied by adverse effects such as nephrotoxicity, neurotoxicity and hyperglycaemia [2]. Recent studies have demonstrated the presence of high levels of eosinophil infiltrates in the gastrointestinal tract following liver transplantation, resulting in the development of eosinophilic disease, a consequence which, although is very important, is underrecognised. These conditions include eosinophilic oesophagitis (EO), eosinophilic gastroenteritis (EG) and eosinophilic colitis (EC). In this review, we discuss the eosinophilic GI disorders associated with immunosuppression after liver transplantation to clarify their diagnosis and treatment.

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The Biology of Eosinophils

First discovered in 1879 by Paul Ehrlich, their name derives from the Greek word “eosin,” meaning “dawn,” reflecting their unique granule populations, consisting of unicompartmental primary granules, bicompartamental secondary granules comprised of a matrix and crystalloid core, and lipid bodies [7]. Eosinophils are ultrastructurally distinguished by their unique granule populations, consisting of unicompartmental primary granules, bicompartamental secondary granules comprised of a matrix and crystalloid core, and lipid bodies [7]. Following an 8-day period of maturation, eosinophils are released into the circulation, a process aided by cytokines, cellular adhesion molecules and eotaxin, an eosinophil-specific chemokine [8]. Only a small proportion of the mature eosinophils remain in the peripheral circulation, with the majority of eosinophils being found in tissues, such as the spleen, lymph nodes, the thymus gland and, of course, the bone marrow, as well as physiologically migrating to the lower gastrointestinal tract (excluding the oesophagus), uterus and mammary glands in the healthy individual. Interestingly, their physiological role in these organs is largely unknown. The half-life of a mature eosinophil is 8-12 hours [7,9].

Eosinophils produce inflammatory mediators including platelet activating factor (PAF), leukotriene C4 and prostaglandins that influence vascular smooth muscle tone and permeability and aid in chemotaxis [10]. Within their unique granule populations and secretory vesicles, eosinophils contain preformed chemokines and cytokines, allowing cell-cell contact thus facilitating regulation, amplification or repair of inflammation. Following an immune stimulus, eosinophils will degranulate, releasing four cationic proteins; major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO). These highly noxious substances are capable of inducing tissue damage and destruction, which may explain the development of eosinophil-associated diseases [4].

Often found in increased numbers at sites of allergic inflammation, eosinophils have been closely linked with allergy and atopy. Allergy and atopy are commonly incorrectly used in substitute for one another and therefore, for purposes of clarity, they have been defined in the following. Allergy is a hypersensitivity reaction by the immune system to an external stimulus or allergy-inducing antigen, known as an allergen. Allergy may be cell-mediated or antibody-mediated, the majority of cases falling under the latter category. Antibody-mediated allergies are most commonly caused by an antibody of the IgE isotype, while non-IgE-mediated allergies are often caused by an antibody of the IgG isotype. Atopy may be defined as the personal and/or familial tendency, usually in childhood or adolescence, to produce IgE antibodies in response to common allergens that are otherwise harmless in non-atopic individuals [11,12]. Atopic diseases include atopic eczema, allergic rhinitis (hayfever), asthma and food allergy. A study by Bischoff and Ulmer (2007) demonstrated the presence of eosinophil granule proteins in the stool samples of children with food allergy following food challenge, showing their involvement in allergy [13]. EGID has also been associated with allergy and atopy; 80% of patients with EGID are atopic and 50% of patients with gastrointestinal allergy are positive for tissue eosinophilia [14].

Immunosuppression in Liver Transplantation

Immunosuppressive therapy is an essential component of the transplantation process in preventing graft rejection. Current immunosuppressive drug regimes involve the use of calcineurin inhibitors (tacrolimus and cyclosporine A), glucocorticoids (prednisone), inhibitors of inosine monophosphate dehydrogenase (IMPDH) (mycophenolate mofetil (MMF)), purine analogues (azathioprine), “target of rapamycin” (TOR) inhibitors (sirolimus and everolimus), mono- and polyclonal antibodies (basiliximab and anti-thymocyte globulin, ATG) [15-17]. Each patient requires different levels of immunosuppression to maintain normal graft function and prevent rejection. Inevitably, this results in varying side effect profiles. In order to reduce the side effect burden, a combination of immunosuppressant drugs are developed, providing optimum immunosuppression with minimal side effects. This is known as tailored immune suppression [18].

The mainstay of immunosuppression for liver transplantation involves the use of the calcineurin inhibitors, tacrolimus and cyclosporine A (CsA) [19]. Despite their structural differences, the mechanism of immunosuppression is similar in both drugs, involving inhibition of the IL-2-mediated T-cell activation. Tacrolimus has replaced CsA as the immunosuppressant drug of choice in liver transplantation as it has a more favourable side-effect profile and is a more potent inhibitor of IL-2 synthesis [20,21]. Since its introduction, several randomised trials have been conducted to assess its efficacy demonstrating the beneficial use of tacrolimus as a prophylactic therapy for organ rejection, especially in cardiac, liver and renal transplants.

Tacrolimus is an 822 kDa macrolide immunosuppressant first discovered in 1984 as a product of the soil bacterium Streptomyces tsukubaensis. Developed as an anti-rejection drug for use in solid organ transplantation, tacrolimus has been proven to significantly reduce rates of both acute and chronic rejection, showing particular therapeutic value in refractory organ rejection [22].

Tacrolimus inhibits the calcineurin-dependent pathway of T cell activation. Tacrolimus binds to the immunophilin, FKBP12, forming the FKBP12-tacrolimus complex, responsible for inhibiting calcineurin, a serine-threonine phosphatase involved in T lymphocyte activation [23]. CsA, in comparison, binds to the immunophilin, cyclophilin (CpN), to induce calcineurin inhibition. Cyclophilins are distributed throughout the body, which may account for the wider range of side effects seen with CsA use over tacrolimus [20]. Inhibition of calcineurin prevents dephosphorylation of nuclear factor of activated T-cells (NFAT), resulting in decreased expression of cytokines such as IL-2, thereby suppressing T cell activation and proliferation (Figure 1) [21,24].

Several theories have been proposed to explain the link between tissue eosinophilia and immunosuppressive therapy for liver transplantation. The interaction between the T-cell receptor (TCR) and allo-antigen stimulates the differentiation of naïve CD4+ T cells into Type 1 or Type 2 T helper cells (Th1 or Th2 cells respectively). Th2 cells produce interleukins (IL)-4 and IL-13, cytokines involved in the IgE-mediated allergic response, and IL-5, an essential component in not only the production of eosinophils, but the eosinophil-mediated allergic response. Th1 cells secrete IFN-γ, which, through inhibition of IL-4 and IL-13, counteracts the effects of Th2-derived cytokines...
thus preventing allergic inflammation [25]. For immune homeostasis to be maintained, it is essential that the Th1 and Th2 cell pathways remain in equilibrium. It has been postulated that the use of tacrolimus and cyclosporine A in liver transplantation may cause an imbalance of Th1 and Th2 responses, blocking the Th1 pathway and favouring the Th2 cell pathway [26]. Through up-regulation of the cell adhesion molecule, VCAM-1, on endothelial cells, Th2 cytokines IL-4 and IL-13 promote production of eotaxin, which, together with IL-5, recruits and activates eosinophils to the site of allergic inflammation (Figure 2) [23,27]. Therefore, tacrolimus and CsA might be expected to promote imbalanced Th2 responses to allergens.

EGID Following Immunosuppression for Liver Transplantation

Gastrointestinal complications are common following liver transplantation and often present with non-specific symptoms. They include infection, surgical adhesions, post-transplant lymphoproliferative disease, food allergy and EGID. There is overlap between the latter two with the use of tacrolimus, as it inhibits mucosal cellular energy production within the intestine causing impairment of the intestinal barrier and as a consequence, increases gut permeability and exposure to antigens [28]. EGID is a term used to describe a group of inflammatory gastrointestinal disorders, characterised by inappropriate eosinophil infiltration and symptoms that affect one or more parts of the GI tract, in the absence of extra-intestinal causes [29]. Among these disorders are eosinophilic oesophagitis (EO), eosinophilic gastroenteritis (EG) and eosinophilic colitis (EC), all of which present with non-specific symptoms and therefore, it has been difficult to distinguish EGID from other gastrointestinal complications of liver transplantation.

**Eosinophilic Oesophagitis (EO)**

The oesophagus is devoid of eosinophils in the healthy individual, but they may migrate here during inflammation. Eosinophilic Oesophagitis (EO) is defined as eosinophil-induced inflammation that is limited to the oesophagus. The peak onset of EO has been identified as 30-40 years, although it can affect adults and children of all ages [30]. A population-based study of children with EO revealed an incidence of 1 in 10,000 and a prevalence of 4.3 and 2.5 out of 10,000 (0.043% and 0.025% respectively) in adults and children respectively, with males three times more likely to suffer than females [31,32]. EO has been associated with liver transplantation following an audit by Noble et al. that verified the presence of EO in 4 of 130 subjects (3% prevalence, one hundred times that of the non-transplant population with EO) who underwent liver transplantation and who were maintained on immunosuppression via either tacrolimus or CsA [33]. Time scale from transplant to diagnosis of EO ranged from 8 months to 9 years 5 months post-transplant, with one child having been diagnosed with EO prior to liver transplantation and relapsing 1 year post-transplantation [33]. Risk factors for EO include male gender, Caucasian race, younger age (<50 years), asthma and food allergies, with age being the greatest risk factor, increasing the relative risk by a factor of 9.5 [34-36].

Definitive diagnosis of EO is based on clinical presentation, endoscopic results and histological features following tissue biopsy. This condition commonly manifests as gastro-oesophageal reflux disease (GORD), with 70% of patients presenting with a chronic, non-progressive dysphagia for solids, with or without food impaction, followed by GORD. Other clinical features include vomiting, haematemesis, weight loss and, in some cases, failure to thrive. The latter symptom is commonly seen with paediatric EO, often accompanied by abdominal pain and GORD [30]. EO is associated with a 4-5 fold
increased incidence of atopic disease compared with that of the general population [37]. Oesophageal furrows seen on endoscopy have been associated with active EO disease, but there are no endoscopic features pathognomic for EO [38]. Furthermore, in some cases, endoscopy may appear normal, despite clinical presentation, but the disease may still be present and thus, biopsy from mid to upper oesophagus must be conducted regardless of endoscopic outcome in order to assess histological involvement, a critical component in the diagnosis of EO.

Histological features include mucosal thickening with papillary lengthening and hyperplasia of the basal layer [38]. Eosinophil number is the most important histological factor in this disease, with much deliberation in the literature as to the threshold number of eosinophils per high power field (HPF) for diagnosis of EO to be given. Generally, an eosinophil count of ≥15 signifies EO, but this threshold may be increased to 20 in order to eliminate any overlap with GORD [31,33,39-42]. The sensitivity of oesophageal biopsy for diagnosis of EO may be maximised by increasing the number of samples from one to five with a sensitivity increment of 45% from 55% with one sample to 100% with five samples (Figure 3). Other histological features include superficial eosinophil aggregates and eosinophilic microabscesses [43]. Peripheral eosinophilia, although seen in one third to one half of patients with EO, is not necessary for diagnosis [44].

EO is known to be unresponsive to standard, and in some cases aggressive, treatment for reflux disease, despite its clinical similarity [33]. First-line treatment of EO is topical corticosteroids in the form of 880-1760 µg swallowed fluticasone per day for 6-8 weeks, which has been well tolerated. Relapse with this disease is common with a recurrence rate of approximately 50-60% at 1 year and subsequent development of oral or oesophageal candidiasis in 20% of EO patients on steroid treatment [45]. Oral prednisone may provide an alternative to fluticasone, but with a wider side effect profile. Other therapies include a six-food dietary elimination (milk, wheat, eggs, soya, nuts, seafood), montelukast (LTD4 receptor antagonist) and mepolizumab (anti-IL-5 monoclonal antibody). Montelukast has been associated with high rates of recurrence within three weeks of cessation of therapy [40]. An uncontrolled trial of mepolizumab demonstrated improved symptoms, endoscopy results and peripheral eosinophilia, overall producing reports of better quality of life [46]. Introduction of mepolizumab therapy also allows for reduction of concurrent steroid therapy by 50% [46].

**Eosinophilic Gastroenteritis (EG)**

Eosinophils are found in the lower gastrointestinal tract in the normal, healthy patient [40]. Romero et al. studied 54 recipients of 57 liver transplants over a study period of 3 years, demonstrating peripheral eosinophilia of ≥10 in 28%, as well as six patients out of 23 with gastrointestinal symptoms, whom, on subsequent gastrointestinal endoscopy and biopsy, were found to have eosinophilic gastroenteritis (EG) in addition to peripheral eosinophilia (Figure 4) [19]. Duration of liver transplantation at diagnosis of EG was not provided, therefore it is difficult to determine a pattern of EG development in these cases.

First described by Kaisjer in 1937, EG is a very rare condition for which there have been reports of less than 300 cases since its discovery [47,48]. The lack of literature on this condition means that it is difficult to assess the true frequency of EG. As with EO, EG predominantly affects adults in the third and fourth decades, but onset may occur at any age. The sex distribution of EG slightly favours the male sex such that males are 1.4 times more likely to be affected by the disease than females. The disease can be classified into three types under the Klein classification which divides the disease according to the layer of intestinal wall that is affected, namely mucosal, muscular and subserosal [49]. Mucosal is the most common type, comprising approximately 57.5% of cases, where the equivalent percentages for
muscular and subserosal diseases are 30% and 12.5% respectively [50]. Mucosal disease can be defined as disease affecting the mucosal layer with mucosal eosinophilic infiltration with no muscular infiltration, obstruction or eosinophil-associated ascites [47]. The symptoms of mucosal type EG include vomiting, diarrhoea, GI bleeding, iron deficiency anaemia, malabsorption and failure to thrive, many of which are also seen with inflammatory bowel disease. Muscular type EG can be defined as disease of the muscle layer with complete or incomplete bowel obstruction and/or eosinophilic infiltration of muscular layer without ascites. Symptoms of this type resemble obstructive disease. Subserosal disease is defined as disease affecting the subserosa with eosinophilic infiltration of the gut and eosinophilic ascites. Symptoms include ascites, high peripheral eosinophilia and possible peritonitis. Risk factors for EG include younger age at transplantation, increased frequency of rejection episodes, tacrolimus-based immunosuppression and detectable EBV load [19].

Non-specificity of EG symptoms means that diagnosis is difficult. Peripheral eosinophilia, although closely linked to EG, is not necessarily present in all patients. Furthermore, a normal eosinophil count may be present in up to one quarter of EG sufferers [50]. 70% of cases of EG occur in those with a personal or family history of atopy and allergy [51].

Aside from eosinophilic infiltration, crypt hyperplasia may be the only diagnostic feature on histological analysis [52]. Disease occurrence has been associated with more frequent rejection episodes, younger age, tacrolimus-based immunosuppression and detectable EBV load [8,19].

Thickening or stenosis of gastric or small bowel folds on radiographical imaging may help to diagnose muscular EG [53]. Non-specific endoscopic features of EG include thickened folds, erythema and nodularity, with increased eosinophil numbers being found following endoscopic biopsy, however there remains insufficient data for a diagnostic threshold to have been identified. Helminthic parasitic infection, tuberculosis and malignancy must be excluded as causes of secondary eosinophilia [40].

Corticosteroid therapy is the mainstay of treatment for non-obstructive EG, however as yet, no therapeutic trials have been conducted given the low frequency of cases and therefore, its efficacy over other therapeutic options is has yet to be established. Studies have shown that corticosteroid therapy is most effective for subserosal EG with an optimum dose of 20–40 mg of prednisone administered for 8 weeks. Budesonide is a promising alternative steroid with fewer side effects [54]. Alternative treatment options include elimination diets, particularly for mucosal EG, montelukast and octreotide. Octreotide, an analogue of somatostatin, increases intestinal absorption and decreases intestinal secretions, which may account for its ability to provide symptomatic relief in EG [55].

Disease relapse is common following cessation of medication in
EG. Immunosuppressant drugs such as azathioprine or mycophenolate mofetil may provide alternative to corticosteroids, but this has yet to be shown in the literature.

**Eosinophilic Colitis (EC)**

Eosinophilic colitis (EC) is a rare complication following liver transplant. First reports of EC emerged in 1979, but there have since been very few cases documented [56]. EC commonly presents with diarrhoea which may be bloody and other constitutional symptoms such as weight loss, anorexia or abdominal pain. Adults with EC often present differently to children with this condition, displaying more obstructive symptoms on presentation such as caecal volvulus or intussusception. Peripheral eosinophilia may also be raised, as with EO and EG, however lack of reports means that it has been difficult to identify a diagnostic cut off value for blood eosinophil number. Eosinophils may also be present in stool samples. Non-specific features of inflammation on endoscopy are similar to those seen with EG. Additional features may include patchy oedematous changes, which disrupt the vascular supply, and superficial ulcers. Histological analysis of rectal biopsies reveals eosinophilic infiltrates, with eosinophils present in the lamina propria, muscularis propria and subserosal layers (Figure 5) [56].

EC has been associated with tacrolimus immunosuppression for liver transplant, following a study of 38 paediatric liver transplants, which demonstrated the presence of EC in 37% rectal biopsies of children maintained on tacrolimus. In the same study, 50% of patients who developed EC also had elevated food-specific IgE levels. Replacing tacrolimus with CsA provided symptomatic relief and reduced mucosal eosinophilia [23,29]. Diagnosis of EC was made between 3 and 10 months post-transplantation, with risk factors identified as peripheral eosinophilia and EBV PCR seroconversion two months post-transplantation (Table 1) [29].

Dietary elimination of any offending food allergens has been indicated in the treatment of childhood EC, leading to resolution within days.

As yet, no therapeutic trials have been conducted to assess steroid treatment for EC, therefore treatment is similar to that of EO and EG entailing topical or systemic corticosteroids such as 20–40 mg prednisone or 9 mg budesonide for 1–2 weeks. Montelukast 10–40 mg has been suggested in the treatment of EC following positive results of its administration in children with duodenal eosinophilia, such as reduction in peripheral eosinophilia and improvement of symptoms [56].

Prognosis for childhood EC is good, with children being able to re-introduce eliminated dietary proteins without problems after few years of remission. The disease is likely to become chronic in adults, presenting intermittently throughout their lifetime (Table 1).

**Conclusion**

EGID is an important, but under recognised complication following immunosuppression for liver transplantation. In the liver transplant population, prevalence of EGID is up to one hundred times greater than the non-transplant population, making it a significant contributor to post-transplant morbidity. They affect individuals of all ages, favouring those in the third and fourth decades, as well as males over females. There is a strong association between these conditions and calcineurin inhibitors, tacrolimus and CsA, with tacrolimus imposing a greater risk for the development of these disorders.

Symptoms of EGID are not only debilitating for the patient, but also highly non-specific making diagnosis of EGID difficult and furthermore, differentiation between EO, EG and EC an even greater challenge. Clinically, EGID commonly manifests with gastrointestinal symptoms including dysphagia, vomiting, haemetemesis, weight loss, anorexia, abdominal pain, diarrhoea and failure to thrive in children. Diagnosis depends on endoscopic and histological findings.

In patients presenting with these symptoms, there must be a high index of suspicion for EGID, prompting further investigation through upper and lower endoscopy and histological analysis.

The mainstay of treatment for EO, EG and EC is systemic steroid therapy, however some specific therapies have been suggested including biologics such as mepolizumab (anti-IL-5 monoclonal antibody) for EO, octreotide (somatostatin analogue) for EG and montelukast (LTD4 receptor antagonist) for all three conditions. Empirical dietary elimination may also provide symptomatic relief.

With regards to prognosis, relapse occurs in the majority of EO and EG patients, whereas remission occurs within days in children with EC. Adult EC appears to run a more chronic course, occurring intermittently throughout the patient’s lifetime.

Prophylactic treatment may be useful in patients at higher risk of developing EGID, such as those with a history of atopy, and in particular, food allergy. EGID is a complication of immune suppression particularly of the drugs that are the mainstay of current anti-rejection therapy. It is quite common and likely to impact on the quality of life of the patient. Modification of the immunosuppressive regime can contribute to reducing risk of relapse and treating active or refractory episodes. Therefore, in patients suffering from EGID, management...
of their immune suppression becomes important in control of the condition rendering EGID a key factor in the design of their tailored immunosuppression.

**References**


**Table 1:** A comparison of eosinophilic gastrointestinal disorders following immunosuppression for liver transplantation.