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**Implications of Eosinophilia in the Normal Duodenal Biopsy
– an Association with Allergy and Functional Dyspepsia**

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Implications of Eosinophilia in the Normal Duodenal Biopsy – an Association with Allergy and Functional Dyspepsia

Running head: Eosinophils in the duodenum

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11 **histopathology, inflammation**
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SUMMARY

Background: Allergy and functional gastrointestinal disorders have been associated with eosinophilia in duodenal mucosa.

Aim: To assess the prevalence of eosinophilia in duodenal biopsies of patients attending for oesophogastroduodenoscopy and delineate associated clinical conditions.

Methods: 155 patients (mean age 55 years, 59% females) with normal duodenal biopsies were randomly selected for audit from histopathology files. Eosinophil counts in 5 high power fields (HPFs) were assessed. Records were analysed for symptoms, diagnosis and medications; patients divided into 5 groups based on upper gastrointestinal (UGI) symptom profiles, including a control group of those without predominant UGI symptoms. The prevalence of duodenal eosinophilia (defined as $>22/5\text{HPFs}$ *a priori*) was calculated.

Results: In the control group the mean duodenal eosinophil count was 15/5HPFs; prevalence of duodenal eosinophilia 22.5%. In postprandial distress syndrome (PDS) both mean eosinophil counts (20.2/5HPF, $p<0.04$) and prevalence of duodenal eosinophilia (47.3%, $p<0.04$) were significantly higher. Duodenal eosinophilia was significantly associated with allergy (OR 5.04, 95% CI 2.12-11.95, $p<0.001$). There was no association with irritable bowel syndrome or medications.

Conclusions: Subtle duodenal eosinophilia is relatively common in routine oesophogastroduodenoscopy and previously overlooked; it is associated with allergy and may indicate a hypersensitivity mechanism in some patients with PDS including early satiety.

INTRODUCTION

The eosinophil is a characteristic bone marrow derived circulating granulocyte with diverse functions; it hosts an array of effector mechanisms important in normal physiology throughout the gastrointestinal (GI) tract of healthy subjects¹. The only part of the GI tract normally free of eosinophils is the squamous epithelium of the oesophagus¹. The normal function of the eosinophil in host defence and immune regulation is carried out via effector secretory granules, which effect antigen presentation, cytokine release, mast cell activation, and immune tolerance². Established in their position in host defence against helminths^{3,4}, eosinophils also play an important role in combating bacterial and viral infections^{1,5}. Gastric eosinophilia occurs in *H. pylori* infection⁵. Dysregulation of eosinophils also occurs in allergic disease, parasite infestation, tumours, drug reactions and hypersensitivity¹.

Small bowel biopsies from patients with asthma and allergic rhinitis show features in common with the inflammatory reaction observed in the airways, with accumulation of eosinophils⁶. Gastrointestinal symptoms are also significantly more common in patients with asthma and allergic rhinitis⁷. Recently, eosinophils have been implicated in contributing to functional gastrointestinal disorders (FGIDs)^{8,9}. FGIDs, including irritable bowel syndrome (IBS) and functional dyspepsia (FD) are defined by chronic abdominal symptoms not associated with known structural or biochemical pathology^{5,9,10}. Definitive criteria for FGIDs are arguably problematic owing to the wide spectrum of manifestations and as a result, FGIDs are grouped by symptoms, and there is a considerable overlap of these disorders based on current classification schemes^{11,12}.

At endoscopy duodenal biopsies are usually taken to exclude coeliac disease, but recent studies⁶⁻⁹ suggest that duodenal eosinophilia may be a marker for atopy, allergy and possibly functional dyspepsia, but not irritable bowel syndrome (IBS)⁸ Eosinophils are

1
2
3 easily visualised on sections stained by haematoxylin and eosin (Figure 1) and therefore
4
5 can be counted by histopathologists without the need for special stains.
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9 The aim of this study was to audit duodenal biopsies previously reported as normal
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11 to assess eosinophil counts and correlate these with review of the clinical records, to
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13 ascertain if these cells may be a biomarker for disease states.
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METHODS

This was a retrospective audit of patients seeking medical care who had one or more upper gastrointestinal endoscopies with duodenal biopsy at St Mary's NHS Trust during October 2004 -November 2008. A randomised list of 155 patients was generated from the histopathology files for all duodenal biopsies coded as normal in this time span. The clinical history and results of investigations were extracted from patient medical records.

Clinical histories were analysed in detail for a range of symptoms, including symptom group definition based on a validated abdominal symptom questionnaire^{13, 14}. Additionally, all basic demographic information and a detailed audit of drug history for all medication at time of OGD or referral were recorded.

Upper GI symptoms

We sought to determine the distribution of eosinophils in the context of upper GI symptoms. Patients were categorised into the following 5 subpopulations based upon the presence and pattern of upper GI symptoms:

Group A: Patients with oesophageal symptoms, including reflux +/- retrosternal non-cardiac chest pain. This group includes patients with GORD and functional oesophageal disorders (Rome III A1-4)¹².

Group B: Patients with symptoms of postprandial distress syndrome (ROME III B1a)¹², including early satiety and postprandial bloating.

Group C: Patients with nausea and vomiting (Rome III B3)¹², in the absence of abdominal pain, oesophageal symptoms or postprandial distress syndrome.

Group D: Patients with abdominal pain, in the absence of postprandial distress syndrome, altered bowel habit or reflux like symptoms

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3 **Group E:** Control group: patients without prominent upper GI symptoms. This group
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5 comprised patients with IBS like symptoms (abdominal pain in association with a change
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7 in bowel habit), isolated diarrhoea, and asymptomatic iron deficiency anaemia. (See
8
9 Figure 2)
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12 This was a retrospective study which fulfilled criteria for audit and no study driven clinical
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14 intervention was undertaken. In keeping with national and local Research and
15
16 Development guidelines this study did not require formal approval from the Local
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18 Research Ethics Committee as it was a retrospective audit of service provision.
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21 22 **Oesophogastroduodenoscopy and Histopathology**

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24 All endoscopies were carried out at St Mary's NHS Trust. Two independent, blinded
25
26 observers assessed duodenal pathology for villous architecture; the intraepithelial
27
28 lymphocyte (IEL) count and any other pathology was also noted. *H. pylori* infection at time
29
30 of biopsy was defined as a positive histological finding of bacteria on gastric sections
31
32 stained with the Gimenez stain¹⁵. Where gastric biopsies were not available, positive
33
34 status was determined by result from diagnostic tests, either the C¹³ urea breath test, or a
35
36 CLO test taken at OGD.
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40 The eosinophil count was performed in 5 randomly selected high power fields (HPFs)
41
42 magnification x40 across the biopsy (Figure 1). Normal was defined *a priori* as <22/HPFs
43
44 based on a control group selected from 1001 community subjects in a previously
45
46 published study⁵ and normal values were calculated in the results of the biopsies from
47
48 patients in the control group E above. The sum, mean and median of number of
49
50 eosinophils per 5HPFs was calculated for each sample. As an internal control
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52 (intraobserver variation), the eosinophil count for the first 20 biopsies was repeated by the
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54 same observer. For external control (interobserver variation), all biopsies were re-counted
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56 by a second independent, blinded observer.
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Statistical analysis

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3 A univariate model assessed eosinophilia as a discrete predictor for pre-defined clinical
4 conditions. Association was identified by calculation of odds ratios with 95% confidence
5 intervals, and significance determined by Fishers Exact Probability test. Eosinophil sum
6 as a continuous predictor was assessed by student's t test for non parametric data,
7 determining significance of difference in median value of eosinophil count in subgroups,
8 assumed where $p = <0.05$. Inter-observer concordance for eosinophil counts was
9 assessed by un-weighted kappa for categorical data. All p values calculated were two-
10 tailed.
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RESULTS

Referrals

The majority of patients (59% female, mean age 55 years) attending endoscopy were referred from general practice (67%). The remainder were from outpatient clinics, in-patient ward referral and emergency OGD including the accident and emergency department. Indications for OGD included predominant upper gastrointestinal symptoms in 42%, weight loss (41%), iron deficiency anaemia (35%) and diarrhoea (34%). Patients were of diverse ethnicity, 72% were from the UK and other EU countries, 19% from Asia and the Middle East and 9% from Africa, the Caribbean and South America.

Upper Gastrointestinal Symptoms

Patients were categorised to 5 groups and categorised according to upper gastrointestinal symptom profiles and included a control population of patients without prominent upper gastrointestinal symptoms (Table 1). It was unsurprising to find that this was the largest subgroup of patients in this study, since duodenal biopsies are commonly acquired in routine clinical practice for suspected coeliac disease. In this group 53/89 (60%) of patients had diarrhoea. Eosinophil scores are summarized in Figure 3.

Table 1

Group	n=	Mean eos count	p vs. control (t-test)	SEM	No. of patients with eosinophil count >22	p vs. control (Fishers exact test)
A	12	14.3	0.82	2.75	3/12 (25%)	1.0
B	19	20.2	0.038	2.72	9/19 (47.4%)	0.04
C	6	17.3	0.56	5.68	1/6 (16.7%)	1.0
D	28	15.4	0.83	1.68	9/28 (32.1%)	0.32
E	89	15.0	-	0.98	20/89 (22.5%)	-

A: Rome III A1-4, (n= 12) B: Rome III B1a, (n=19) C: Rome B3, (n=6) D: abdominal pain (no PDS, altered bowel habit or reflux like symptoms) (n=28) and E: controls, those without prominent upper GI symptoms, (n=89).

Group B versus controls OR=3.1 (95% CI 0.98-9.85).

Histopathology and eosinophilia

All patient histology reports were coded as normal with no overt duodenal pathology.

None of the subjects in this audit were diagnosed with coeliac disease. Although one patient had a raised tissue transglutaminase antibody, there was no abnormality at OGD in this patient; no change in villous architecture and the IEL count was normal at 6 per 100 enterocytes. 45% had duodenal eosinophil counts >15/ 5HPFs, as in the control group without upper GI symptoms and 27% had duodenal eosinophil counts >22/5HPFs.

There was no significant association with age ($p=0.09$), gender, ($p=0.5$) or *H. pylori* status ($p=0.6$)

Atopy and Allergy

Duodenal eosinophilia was significantly more common in patients with a history of allergy (OR 5.04, 95% CI 2.12-11.95, $p < 0.001$). The overall prevalence of allergy in the study population was 20%, and just under half of all patients with duodenal eosinophilia $>15/5\text{HPFs}$ (44%) were in this group. Twenty three had asthma; a further 8 reported hay fever and the remainder included a past medical history of eczema ($n=11$), urticaria ($n=2$), and wheat or milk food allergy ($n=7$). Whilst eosinophilia *per se* was significant in these patients, they did not have a significantly higher association of any upper GI symptom with eosinophilia ($p=0.3$), however group B (patients with postprandial distress syndrome) were significantly more likely to report a history of allergy versus those without upper gastrointestinal symptoms (OR 4.82, CI 1.6-14, $p=0.004$). The eosinophil scores are summarised in Figure 4.

Medications

In these patients, 70% were on one or more medications, including non-steroidal anti-inflammatory drugs (25%), proton pump inhibitors, (35%), (61% on both) antihypertensives, (37%), statins (20%) iron (16%), sulphonylureas, (10%) steroids, (7%) and other drugs (32%) including calcium supplementation, antiviral and immunosuppressive therapy, antiarrhythmic agents, diuretics, antidepressants, antiemetics, the oral contraceptive pill, hormone replacement therapy and compound analgesics) at the time of biopsy or in the preceding time from referral. No significant association with eosinophilia was found with any medication ($p=0.1$) or separate class of drugs (NSAIDs, $p=0.23$, PPIs $p=0.78$) Drug allergy (principally to penicillin) was declared by 17%, but no significant association with eosinophilia was found ($p=0.62$) in these patients.

Seasonal variation

No significant difference was seen between eosinophil counts in biopsies taken in the autumn/ winter seasons and the spring/ summer seasons (OR 1.1, CI 0-2.9, $p=0.8$).

Similarly in allergy subjects, there was no significant difference either by season (OR 0.8 CI 0.2-2.7, $p=0.7$).

Reliability of Histopathology

Assessment of interobserver concordance in eosinophil counts showed “almost perfect agreement” with an unweighted kappa value of 0.81 (95% CI 0.65-0.97). The first 20 eosinophil counts were repeated and re-counted after all 100 counts has been performed; the intra observer unweighted kappa was 0.53 (95% CI 0.15-0.89) and showed moderate agreement, which showed there to be a learning curve in counting eosinophils.

DISCUSSION

Eosinophils are a normal constituent of the gastrointestinal tract, although the question of what constitutes eosinophilia is vexed by very few studies in truly normal controls^{9, 16} and subtle changes in numbers may be significant¹⁷. In the duodenum, eosinophilia has been noted in children and adults with functional dyspepsia, defined as >10/1HPF for children¹⁶ and >22/5HPFs at the base of glands for adults⁵. In this study, we have audited eosinophilia in duodenal biopsies from 155 adult patients attending for OGD, and observed a significant association between duodenal eosinophil numbers and a history of allergy and post prandial distress most notably early satiety.

Previous studies in patients with allergy and atopy have shown that these patients have gastrointestinal symptoms^{6, 7, 18} and that eosinophils may be implicated in functional dyspepsia^{8, 19}. These results strengthen the hypothesis that hypersensitivity underlies gastrointestinal disorders in a subset of patients. It is possible that eosinophils contribute to an altered local cytokine environment, causing visceral symptoms in the upper abdomen. Seasonal variation may be important, as duodenal mucosal eosinophils have been shown to increase in the birch pollen season in one study²⁰ however; we found no significant difference by season of biopsy, which has also been shown in biopsies of colonic mucosa in patients without colitis undergoing cancer screening taken during seasonal elevations in pollen counts²¹.

Early satiety is a classical symptom of dyspepsia^{11, 22}. Our analysis demonstrates a significant association between eosinophils and this symptom although the number included was modest. We have previously also observed that duodenal eosinophilia was specifically linked to early satiety⁵. There were no other causes for eosinophilia in the patients in the present study reporting early satiety; all were *H. pylori* negative, with no parasite infection, malignancy, and inflammation; further, raised IEL counts and coeliac disease were excluded. Of these patients, one had a hiatus hernia, with the remaining

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3 patients all given an inconclusive diagnoses, mostly grouped as functional disorders who
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5 were discharged after further investigation yielded no positive results. Such cases
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7 highlight the possible benefits of recognising eosinophilia in avoidance of 'diagnosis of
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9 exclusion' and fruitless additional uncomfortable investigations. There was a positive,
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11 although not statistically significant link to dyspepsia as a symptom cluster, suggesting
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13 that eosinophilia may be a marker of certain pathologies in these disorders. However, our
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15 study was not powered to assess the link to dyspepsia, and further work is needed to
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17 evaluate the association of duodenal eosinophilia with specific dyspepsia symptoms.
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19 Dyspepsia is a constellation of symptoms, and fruitful work may come from further
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21 investigation of those with early satiety as the predominant complaint rather than placing
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23 these all into the functional dyspepsia category. Early satiety may be due to neurological
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25 dysfunction, which we postulate might be caused by eosinophil mediated axonal necrosis,
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27 as seen in murine eosinophil gastrointestinal disorder models²³ and eosinophil
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29 interference with muscarinic receptors. Major basic protein (MBP) released from
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31 eosinophil degranulation can induce vagal M2 receptor dysfunction increasing smooth
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33 muscle reactivity²⁴. Importantly, eosinophil stimulated T cell activation and mast cell
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35 degranulation releases lipid mediators, leukotrienes, which are a potent stimulator of
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37 smooth muscle contraction¹⁹ (Figure 5).
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43 The lack of association of medication is not perhaps surprising in view of few
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45 reports of drug induced eosinophilic gastroenteritis²⁵. The majority of patients (70%) were
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47 on one or more medications, and no association of eosinophilia noted with any class of
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49 medication. Whilst an association with use of proton pump inhibitors and the emergence
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51 of eosinophilic oesophagitis has been mooted²⁶, duodenal eosinophilia was not
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53 associated with use of this drug in these patients, even though a significant proportion of
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55 patients (35%) were on this therapy.
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59 The audit design had a number of strengths. The randomly generated study
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population of 155 healthcare seeking individuals should be representative of patients

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3 currently reported to have a “normal” duodenal biopsy. The cases provide a diverse range
4 of indications, co-morbidities and symptoms in which eosinophilia was assessed. Based
5 on the histological analyses, the kappa scores for concordance showed excellent
6 reliability. This suggests that despite sampling errors of biopsy tissue, simple quantitative
7 histopathology is a reliable and practical tool for assessing eosinophil counts. There are
8 also limitations to consider. The results may not apply to other referral centres, and more
9 data are needed to confirm the associations observed here. Further population-based
10 normal values also need to be obtained in the UK, as this London population is ethnically
11 diverse. A previous study from Sweden found a higher value of eosinophils, albeit done by
12 the same senior pathologist⁸.

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26 In summary, this investigation suggests that a subtle increase in eosinophils in the
27 duodenal mucosa has important clinical correlations, and this association until now has
28 been an overlooked pathological constituent of the intestinal mucosa. The presence of
29 eosinophilia may indicate a hypersensitivity-mediated disorder that could be amenable to
30 directed treatment. Future studies need to prospectively analyse patients attending OGD,
31 with the aid of a standard questionnaire, to enable further exploration of the role of
32 duodenal eosinophilia.
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Legends for Figures

Figure 1

The characteristic morphological features of eosin uptake in the cytoplasm and bi-lobed nuclei identify eosinophils in H&E stained sections (original magnification x 100).

Figure 2

Flow chart of patient selection

Figure 3

Eosinophil counts in the duodenum

Groups: A: Rome III A1-4, (n= 12) B: Rome III B1a, (n=19) C: Rome B3, (n=6) D: abdominal pain (no PDS, altered bowel habit or reflux like symptoms) (n=28) and E: controls, those without prominent upper GI symptoms, (n=89).

● represents median eosinophil count /5HPFs

Figure 4

Eosinophil counts / 5HPF in the duodenum in patients with allergy

Groups:A: Rome III A1-4, (n= 12) B: Rome III B1a, (n=19) C: Rome B3, (n=6) D: abdominal pain (no PDS, altered bowel habit or reflux like symptoms) (n=28) and E: controls, those without prominent upper GI symptoms, (n=89).

● represents median eosinophil count /5HPFs

Figure 5

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3 Eosinophils are activated by allergens and infection and the TH2 cytokines interleukin (IL)
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5 –5, IL-4 and IL-13. Degranulation products such as nerve growth factor (NGF) have a
6
7 direct action on sensory nerves and major basic protein (MBP) can induce vagal nerve
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9 muscarinic receptor (M2) dysfunction. Platelet aggregating factor (PAF), leukotrienes (LT)
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11 and interleukin 13(IL-13) are also able to act directly on the smooth muscle, increasing
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13 contractility and reactivity. Thus, eosinophil accumulation may cause neurological
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15 dysfunction and evoke visceral symptoms.
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For Peer Review

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Conflicts of interests for this study:

Marjorie M Walker nil

Sormeh S Salehian nil

Claire E Murray nil

Arun Rajendran nil

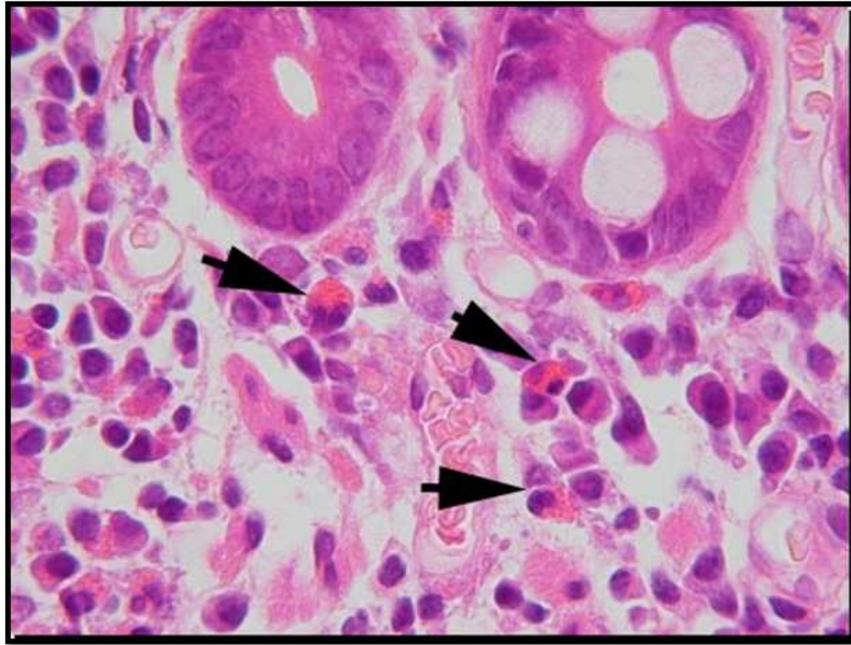
Jonathan M Hoare nil

Rupert Negus nil

Nicholas Powell nil

Nicholas J Talley nil

For Peer Review

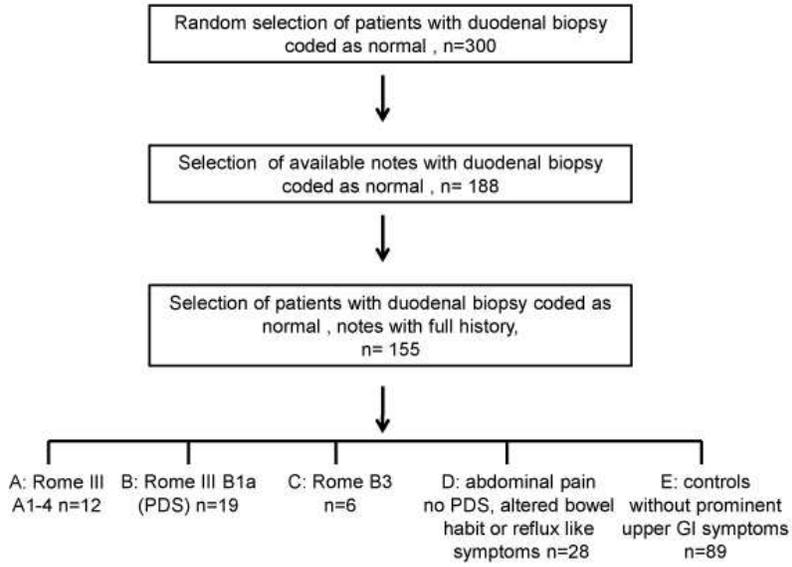


The characteristic morphological features of eosin uptake in the cytoplasm and bi-lobed nuclei
identify eosinophils in H&E stained sections
252x168mm (72 x 72 DPI)

Review

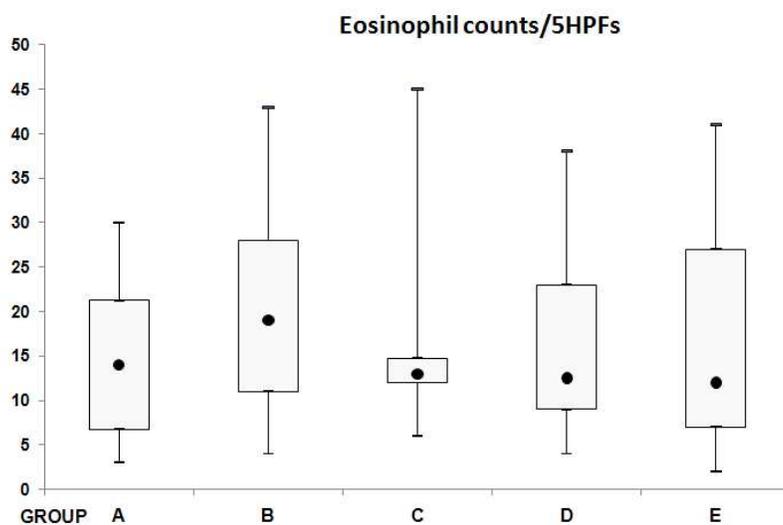
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Flow chart of patient selection
254x190mm (72 x 72 DPI)

Review



27 Eosinophil counts in the duodenum

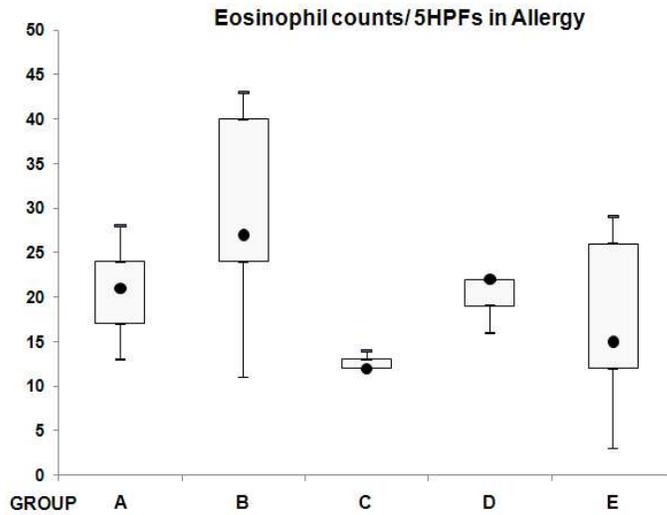
28 Groups: A: Rome III A1-4, (n= 12) B: Rome III B1a, (n=19) C: Rome B3, (n=6) D: abdominal pain
29 (no PDS, altered bowel habit or reflux like symptoms) (n=28) and E: controls, those without
30 prominent upper GI symptoms, (n=89).

31 305x168mm (72 x 72 DPI)

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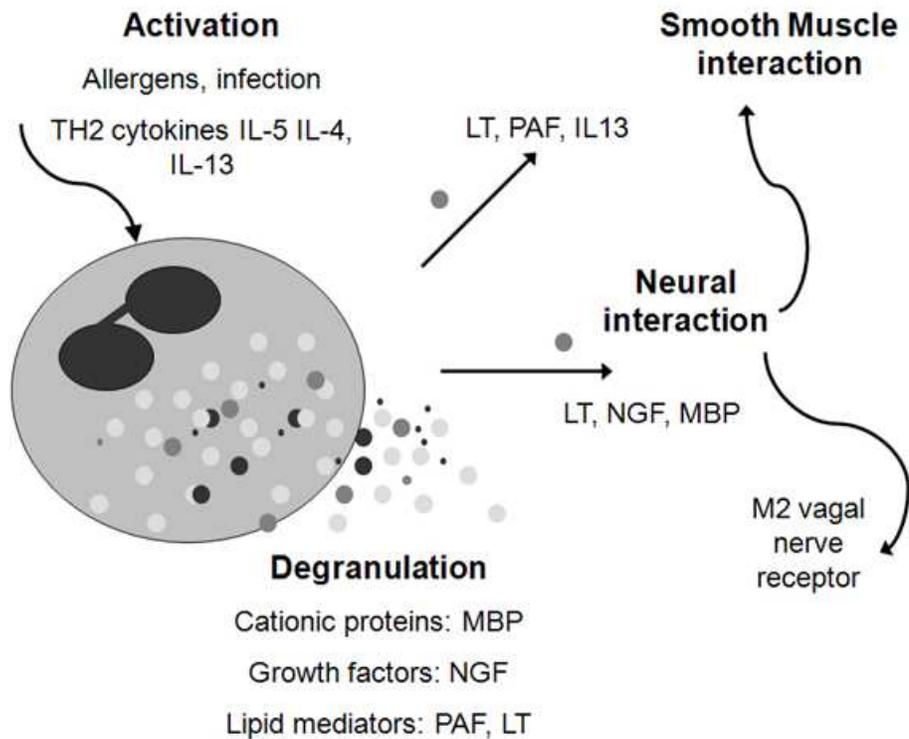
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Eosinophil counts / 5HPF in the duodenum in patients with allergy
Groups:A: Rome III A1-4, (n= 12) B: Rome III B1a, (n=19) C: Rome B3, (n=6) D: abdominal pain (no PDS, altered bowel habit or reflux like symptoms) (n=28) and E: controls, those without prominent upper GI symptoms, (n=89).

305x168mm (72 x 72 DPI)

Review



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Eosinophils are activated by allergens and infection and the TH2 cytokines interleukin (IL) -5, IL-4 and IL-13. Degranulation products such as nerve growth factor (NGF) have a direct action on sensory nerves and major basic protein (MBP) can induce vagal nerve muscarinic receptor (M2) dysfunction. Platelet aggregating factor (PAF), leukotrienes (LT) and interleukin 13(IL-13) are also able to act directly on the smooth muscle, increasing contractility and reactivity. Thus, eosinophil accumulation may cause neurological dysfunction and evoke visceral symptoms

221x167mm (72 x 72 DPI)