Table S1: Quality assessment criteria

|  |  |  |  |
| --- | --- | --- | --- |
| **Criteria** | **0** | **1** | **2** |
| **Are the aims of the study clearly stated?** | Aims not stated | Some aims stated clearly/all aims stated unclearly/not in abstract | All aims stated clearly & included in abstract |
| **Are the inclusion & exclusion criteria for participants included?** | No mention of participant criteria | Only either inclusion or exclusion criteria included | Both inclusion & exclusion criteria included |
| **Are participant demographic characteristics included?** | No reference to participant characteristics | Some reference to participant characteristics | Detailed references to participant characteristics |
| **Are FGIDs classed according to subtype?**  | No classification beyond 'FGID' | Classified into either IBS or FD | Classified into Rome criteria IBS subtypes or FD subtypes |
| **Are any comorbidities acknowledged & controlled for?** | Controlling for comorbidities not mentioned | Comorbidities mentioned but not controlled/some comorbidities mentioned | Comorbidities mentioned & controlled for |
| **Was ethical approval obtained & acknowledged?** | No reference to ethical approval | Ethical approval from unnamed body | Ethical approval gained from a named body |
| **Were power calculations performed?** | No mention of power calculations performed | Power calculation attempted | Study numbers match power calculation |
| **Are statistics appropriate?** | No statistics/inappropriate statistics | Appropriate statistics but no justification given | Appropriate statistics used with justification |
|  **Are p values included?** | No p values included |  p values included, but not all exact/not all p values included  | All p values included with exact values |
| **Are limitations of the study acknowledged?** | No limitations acknowledged | Some but not all limitations acknowledged/acknowledgement of limitations unclear | Limitations acknowledged |
| Total score: \_\_\_\_/20 |

Table S2: Demographic information of studies included in systematic review

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study reference** | **Year** | **Journal** | **Patient population** | **Cohort location** | **Diagnostic Criteria** | **Mean age (years)** |
| Kindt *et al.* | 2009 | Neurogastrol Motil | 32 controls 30 IBS23 FD15 NCCP | Belgium | Rome II | Controls = 30.5 (24.5-44.5) Patients = 40.5 (30.5-50.5) |
| Walker *et al.* | 2009 | Aliment Pharmacol Ther | 48 control51 FD20 IBS-C21 IBS-D | Sweden  | Rome II for FD Rome I for IBS | Controls = 53 (±13.5)FD = 53 (±13.5) IBS = 54 (±14.8) |
| Wang *et al.* | 2015 | Ann Diag Path | 39 controls141 FD | China  | Rome III | Controls = 47.4 (±10.6) FD = 46.2 (±7.9) |
| Du *et al.* | 2016 | Nat Scientific Reports | 24 controls96 FD | China  | Rome III | Controls = 45.2 (±7.2) FD = 47.6 (±11.2) |
| Futagami *et al.* | 2010 | Am J Gastroenterol | 20 controls36 EPS65 PDS 17 PI-EPS18 PI-PDS | Japan | Rome III | Controls = 46.2 (±12.8)EPS = 44.4 (±9.6)PDS = 51.2 (±8.3)PI-EPS = 43.8 (±8.5)PI-PDS = 49.2 (±11.5) |
| Gargala *et al.* | 2007 | World J Gastroenterol | 12 controls 26 FD | France | Rome II | Controls = 42 (19-76)  FD = 44 (18-69) |
| Liebregts *et al.* | 2011 | Am J Gastroenterol | 35 controls 45 FD23 IBS | Australia  | Rome II | Controls = 41.8 FD = 41.8 IBS = 44.2 |
| Talley *et al.* | 2007 | Clin Gastroenterol & Hepatol | 48 controls51 FD | Sweden  | Rome II for FD Rome I for IBS | Controls = 53.4 (13.5)FD = 53.4 (13.5) |
| Hall *et al.* | 2003 | Clin Gastroenterol & Hepatol | 20 controls33 FD/*H. pylori+ve* 29 FD/*H. pylori*-ve 29 inflammatory controls | Ireland | Rome II | Controls = 40 (18-79)FD/Hp+ve = 36 (18-79)FD/Hp-ve = 34 (18-79)Inflam. controls = 33 (21-68) |
| Vanheel *et al.* | 2014 | Gut | 15 controls15 FD  | Belgium | Rome III | Controls = 28.4 (±2.3)FD = 28.9 (±2.6) |
| Ahn *et al.* | 2014 | Dig Dis Sci | 25 controls83 IBS-D49 UC | Republic of Korea | Rome III | Controls = 40 (32-49.5)IBS-D = 32 (25.5-45.0)UC = 42 (29-54) |
| Akbar *et al.* | 2008 | Gut | 22 controls8 IBS-D8 IBS-C7 IBS-A | England | Rome II | Controls = 64 (55-75)IBS-D = 34 (26-57)IBS-C = 59 (49-69)IBS-A = 65 (24-70) |
| Barbara *et al.* | 2004 | Gastroenterology | 22 controls44 IBS (50% IBS-D, 50% IBS-C) | Italy | Rome II | Controls = 32.5 (20-71)IBS = 40.1 (22-75) |
| Bennet *et al.* | 2016 | Am J Gastroenterol | 58 controls41 IBS-C69 IBS-D63 IBS-A/M | Sweden  | Rome III | Controls = 27 (25-34)IBS-C = 35 (26-46)IBS-D = 31 (25-43)IBS-A/M = 28 (24-37) |
| Bertiaux-Vandaele *et al.* | 2011 | Am J Gastroenterol | 33 controls19 IBS-D14 IBS-C15 IBS-A2 IBS-U | France | Rome III | Controls = 57.4 (±14.0)IBS-D = 45.5 (±13.2)IBS-C = 54.7 (±15.7)IBS-A = 49.3 (±15.7)IBS-U = 44.5 (±7.5) |
| Brint *et al.* | 2011 | Am J Gastroenterol | 19 controls26 IBS patients (29% IBS-D, 19% IBS-C, 52% IBS-A/M)29 IBD | Ireland | Rome II | Controls = 41 (28-64)IBS = 36 (21-56)IBD = 37 (22-55) |
| Chadwick *et al.* | 2002 | Gastroenterology | 28 controls77 IBS patients(55% IBS-D, 14% IBS-C, 31% IBS-A) | New Zealand | Rome criteria (no number listed) | Controls = 51 (25-68)IBS patients = 49 (19-79) |
| Chang *et al.* | 2011 | Am J Gastroenterol | 19 male controls22 female controls1 M IBS-C, 15 F IBS-C10 M IBS-D, 5 F IBS-D8 M IBS-A/M, 6 F IBS-A/M | USA | Rome II | Male controls = 42.7 (±2.5)Female controls = 33.0 (±1.9)Male IBS = 43.3 (±2.4)Female IBS = 37.7 (±2.0) |
| Chen *et al.* | 2012 | BMC Gasteroenterol | 20 controls20 PI-IBS18 IBS | China  | Rome III | Controls = 43.74 (±7.2)PI-IBS = 49.71 (±11.2)IBS = 40.52 (±5.2) |
| Darkoh *et al.* | 2014 | PLoS ONE | 40 controls54 IBS16 PI-IBS | USA | Rome II | Controls = 53 (25-86)IBS = 54 (24-79)PI-IBS = 50 (28-66) |
| Dlugosz *et al.* | 2017 | Biomed Res Int | 14 controls7 IBS-D8 IBS-C 7 IBS-M | Sweden  | Rome II | Controls = 42 (22-61)IBS = 39 (18-66) |
| El-Salhy *et al.* | 2010 | Dig Dis Sci | 42 controls23 IBS-D18 IBS-C | Norway | Rome III | Controls = 41 (18-63)IBS = 35 (18-58) |
| Foley *et al.* | 2011 | Gastroenterology | 29 controls20 IBS-D21 coeliac disease | England | Rome III | Controls = 32.3 (±1.7)IBS-D patients = 41.6 (±3.4)Coeliac patients = 41.7 (±3.2) |
| Forshammar *et al.* | 2008 | Scand J Gastroenterol | 11 controls5 IBS-D7 IBS-A | Sweden  | Rome II | Controls = 40 (±7.9)IBS = 35.6 (±13.5) |
| Gao *et al.* | 2013 | Experi & Ther Med | 15 controls12 IBS (no anxiety/dep.)16 IBS (w/ anxiety/dep.) | China  | Rome III | Controls = 38.5 (20-57)IBS = 39 (17-61)IBS (anxiety & dep.) = 37.5 (17-58) |
| Guilarte *et al.* | 2007 | Gut | 14 controls20 IBS-D | Spain | Rome II | Controls = 27.9 (22-53)IBS-D = 32.8 (21-56) |
| Holmen *et al.* | 2007 | Neurogastrol Motil | 26 controls21 IBS-D 2 IBS-C11 IBS-A | Sweden  | Rome II | Controls = 53 (±10)IBS = 40(±13) |
| Ishimoto *et al.* | 2017 | J Clin Biochem & Nutrition | 20 controls17 IBS-D | Japan | Rome III | Controls = 64 (±12)IBS-D = 54 (±19) |
| Lee *et al.* | 2008 | J Gastroenterol Hepatol | 12 controls17 IBS-D13 IBS-C7 IBS-M5 PI-IBS | South Korea | Rome III | Controls = 50.8 (±11.5)IBS = 48.1 (±11.2)PI-IBS = 44.4 (±14.0) |
| Liebregts *et al.* | 2007 | Gastroenterology | 36 controls18 IBS-M17 IBS-C20 IBS-D | Australia  | Rome II | Controls = 37.5IBS patients = 39.5 |
| Macsharry *et al.* | 2008 | Scand J Gastroenterol | 39 controls59 IBS (52% IBS-A, 29% IBS-D, 19% IBS-C)28 IBD | Ireland | Rome II | Controls = 46 (28-64)IBS = 36 (21-56)IBD = 37 (22-55) |
| McKernan *et al.* | 2011 | Aliment Pharmacol Ther | 30 controls9 IBS-D10 IBS-C11 IBS-A | Ireland | Rome II | Controls = 36.24 (±1.835)IBS = 40.9 (±2.034) |
| Ohman *et al.* | 2009 | Am J Gastroenterol | 30 controls26 IBS-D11 IBS-C37 IBS-A | Sweden  | Rome II | Controls = 39 (±10)IBS = 34 (±16) |
| Ohman *et al.* | 2005 | Clin Gastroenterol & Hepatol | 15 controls20 IBS-D4 IBS-C9 IBS-A23 UC | Sweden  | Rome II | Controls = 53 (±8)IBS = 42 (±12)UC = 42 (±11) |
| Ohman *et al .* | 2009 | Neurogastrol Motil | 30 controls26 IBS-D11 IBS-C37 IBS-A | Sweden  | Rome II | Controls = 39 (±10)IBS = 34 (±16) |
| Ohman *et al.* | 2012 | Eur J Gastroenterol Hepatol | 30 controls26 IBS-D11 IBS-C37 IBS-A | Sweden  | Rome II | Controls = 39 (±10)IBS = 34 (±16) |
| O'Sullivan *et al.* | 2000 | Neurogastrol Motil | 7 controls8 IBS-D2 IBS-C4 IBS-A7 inflammatory controls | Ireland | Rome (not defined) | Controls = 44 (±8)IBS = 42 (±11) |
| Rana *et al.* | 2012 | Trop Gastroenterol | 62 controls63 IBS-D | India | Rome II | Controls = 43.5 (25-64)IBS-D = 42.6 (26-65) |
| Seyedmirzaee *et al.* | 2016 | Clin Res Hepatol Gastroenterol | 75 controls34 IBS-D29 IBS-C11 IBS-M | Iran | Rome III | Controls = 37.37 (±12.5)IBS patients = 35.52 (±11.72) |
| Shulman *et al.* | 2014 | J Gastroenterol  | 88 female IBS18 male IBS | USA | Rome III | Female IBS = 39 (±15)Male IBS = 41 (±17) |
| Sohn *et al.* | 2014 | Scand J Gastroenterol | 21 controls22 IBS-D | Korea | Rome III | Controls = 53.4 (±12.0)IBS-D = 50.0 (±15.1) |
| Sundin *et al.* | 2014 | Scand J Gastroenterol | 9 controls10 PI-IBS | Sweden  | Rome III | Controls = 27.7 (20-42)PI-IBS = 41.6 (25-55) |
| Vicario *et al.* | 2015 | Gut | 30 controls49 IBS-D | Spain | Rome III | Controls = 29 (21-64)IBS-D = 35 (18-63) |
| Wang *et al.* | 2007 | World J Gastroenterol | 20 controls20 IBS-D18 IBS-C | China  | Rome III | Controls = 39.9 (±19.5)IBS-D = 48.7 (±16.6)IBS-C = 41.5 (±15.1) |
| Zhen *et al.* | 2015 | Mol Med Report | 20 controls42 IBS-D | China  | Rome III | Female controls = 35.1 (±8.1)Male controls = 37.9 (±7.8)Female IBS-D = 38.1 (±5.9)Male IBS-D = 36.4 (±6.9) |
| Park *et al.* | 2006 | J Gastroenterol Hepatol | 15 controls18 IBS-D | Korea | Rome II | Controls = 41.4 (31-57)IBS-D = 42.6 (25-65) |
| El-Salhy *et al.* | 2012 | Mol Med Reports | 27 controls30 IBS-D20 IBS-C | Norway | Rome III | Controls = 53 (20-65)IBS = 34 (18-62) |
| Lee *et al.* | 2013 | J Neurogastroenterol Motil | 7 controls16 IBS-D | Korea | Rome II | Controls = 49.0 (38-64)IBS-D = 54.6 (24-66) |
| Cremon  *et al.* | 2009 | Am J Gastroenterol | 24 controls12 MC20 UC27 IBS-D21 IBS-C | Italy | Rome II | Controls = 32.0 (±14.9)MC = 38.9 (±18.0)UC (active) = 36.9 (±9.9)UC (remission) =44.6 (±16.6)IBS-D = 43.5 (±13.6)IBS-C = 41.6 (±16.4) |
| Walker *et al.* | 2014 | Human Pathology | 745 subjects representative of general populaton | Sweden | Rome III | 43% male, mean age = 51 |
| Braak *et al.* | 2012 | Am J Gastroenterol | 20 controls15 IBS-D15 IBS-C36 IBS-A | Belgium/The Netherlands | Rome II | Controls = 31 (±3)IBS = 38 (±2) |

Table S3: Studies presenting circulating cytokine findings.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **FGID** | **Study**  | **Year** | **Journal** | **Blood sample** | **Quality score (/20)** | **Circulating cytokine findings** |
| FD & IBS | Kindt *et al.* | 2009 | Neurogastrol Motil | PBMCs. | 13 | No difference in basal IL-6 or IL-10 plasma levels (*p*=0.33) however stimulation (PHA & anti-CD28) increases patient levels of IL-5 and IL-13 with a decrease in IFN-γ production (*p*<0.01).HADs score was correlated with stimulated IL-5, IL-13 and IFN-γ levels (*p*<0.05). |
| FD & IBS (FD focused) | Liebregts *et al.* | 2011 | Am J Gastroenterol | PBMCs.  | 14 | Higher secretion of cytokines TNF-α (*p*=0.02), IL-1β (*p*=0.003), IL-10 (*p*=0.007) from PBMCs of FD patients compared to controls. IL-1β & IL-10 positively correlated with upper abdominal pain, cramps, nausea & vomiting. TNFα correlated with cramps & vomiting (*p*<0.05). IL-6 associated with higher abdominal pain scores.No association with cytokine levels or T cells & fullness or early satiety symptoms. |
| IBS | Bennet *et al.* | 2016 | Am J Gastroenterol | Serum.  | 16.5 | No discrimination between IBS patients or controls possible based on serum level of IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, TNF, FoxP3 (R^2=0.2).More variance between patient cytokine levels within the IBS group than within the control cohort. |
| IBS | Chang *et al.* | 2011 | Am J Gastroenterol | Serum.  | 16.5 | No significant differences in serum levels of IL-1β, IL-6, IL-8, IL-10, IL-12 or TNFα between IBS patients & controls, within males & females (*p*>0.05).Decreased serum IL-10 correlated with higher symptom intensity in the overall patient cohort compared to controls (*p*=0.006).Negative correlation between serum IL-12 & HAD score (*p*=0.007). |
| IBS | Darkoh *et al.* | 2014 | PLoS ONE | Serum.  | 12.5 | Significant increase in serum IFN-γ, IL-1β & TNF-α in patients with IBS compared to controls (*p*<0.05 for all).Significantly higher concentration of CXCL16 in serum & stool of patients with IBS & PI-IBS when compared to controls (p<0.05 for both). |
| IBS | Gao *et al.* | 2013 | Experi & Ther Med | Blood supernatant | 10.5 | Significant increase in blood levels of IL-1β in patients with IBS compared to controls (*p*<0.05).Significant decrease in blood levels of IL-10 in patients with IBS compared to controls (*p*<0.05).IBS-D patients with anxiety & depression had significantly higher levels of IL-1β in blood when compared to IBS-D patients without anxiety & depression (*p*<0.05).IBS-D patients with anxiety & depression had significantly lower levels of IL-10 in blood when compared to IBS-D patients without anxiety & depression (*p*<0.05). |
| IBS | Liebregts *et al.* | 2007 | Gastroenterology | PBMCs | 17.5 | Significantly increased baseline levels of TNF-α, IL-1β and IL-6 in IBS patients compared to controls (*p*<0.17 for all). Subgroup analysis demonstrated these cytokines to only be significantly increased in IBS-D patients (*p*<0.05). Of these, patients with PI-IBSD had increased levels of all cytokines compared to healthy controls (*p*<0.05) but no change when compared to IBS-D patients. IBS patients had higher LPS stimulated IL-6 levels (*p*<0.0166). |
| IBS | McKernan *et al.* | 2011 | Aliment Pharmacol Ther | Plasma.  | 18 | Significantly increased plasma levels of IL-6 (*p*=0.008), IL-8 (*p*=0.028) and cortisol (*p*=0.048) in IBS patients compared to controls. No change in plasma levels of IL-1β, TNF-α, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13 or IFN-γ between IBS patients & controls. No gender differences observed. |
| IBS | Ohman *et al.* | 2012 | Eur J Gastroenterol Hepatol | PBMCs. Serum.  | 14 | No change in serum levels of IL-10, IL-6 or IL-1β.  |
| IBS | Rana *et al.* | 2012 | Trop Gastroenterol | Serum.  | 12.5 | Significant increase in serum IL-6 and TNF-α levels in IBS-D patients compared to controls (*p*<0.05 for both).No difference in serum levels of IL-10 between IBS-D patients and controls (*p*=0.23).No correlation between pro-inflammatory cytokine levels & symptom severity. |
| IBS | Seyedmirzaee *et al.* | 2016 | Clin Res Hepatol Gastroenterol | Serum.  | 14 | Significant increase in levels of IL-6, IL-8 & TNF-α in serum of patients with IBS when compared to controls. This significant increase in all 3 cytokines was maintained when each IBS subtype was compared individually to controls (*p*<0.05 for all).No significant difference between serum levels of IL-6, IL-8 & TNF-α when IBS subtypes were compared to one another (*p*>0.05 for all). |
| IBS | Shulman *et al.* | 2014 | J Gastroenterol  | PBMCs.  | 15.5 | No difference between men & women with IBS in PBMC IL-10 production.  |
| IBS | Zhen *et al.* | 2015 | Mol Med Report | PBMCs.  | 15.5 | Significantly increased levels of TNF-α (*p*<0.001) & IL-8 (*p*=0.007) in IBS-D patients compared to controls.Decreased levels of IL-10 in IBS-D patients compared to controls (*p*=0.047).Positive correlation between symptom score & TNF-α & IL-8 production (*p*<0.001 for both).Negative correlation between symptom score & IL-10 production (*p*<0.001 for both). |

Table S4: Studies presenting tissue cytokine findings.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **FGID** | **Study**  | **Year** | **Journal** | **Samples analysed** | **Biopsy sample site** | **Quality score (/20)** | **Tissue cytokine findings** |
| IBS | Foley *et al.* | 2011 | Gastroenterology | Small intestinal biopsies. | Duodenal biopsies, site not specified | 15 | No statistical difference in mRNA expression of IL-1, IL-6, IL-10 & IL-13 in IBS patients or controls (*p*>0.05 for all). |
| IBS | Bennet *et al.* | 2016 | Am J Gastroenterol | Colon biopsies. | Sigmoid. | 16.5 | No discrimination between IBS patients or controls possible based on mucosal mRNA expression of IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IFN-γ, TNF, FoxP3 (R^2=0.2). |
| IBS | Chang *et al.* | 2011 | Am J Gastroenterol | Colon biopsies.  | Sigmoid | 16.5 | No differences in colonic mRNA expression of IL-1β, IL-6, IL-8, IL-12 or TNF-α between IBS patients & controls, or within males & females (p>0.05).Significant decrease in colonic expression of IL-10 in female IBS patients compared to female controls (*p*=0.007). |
| IBS | Chen *et al.* | 2012 | BMC Gasteroenterol | Colon biopsies | Ascending, descending, rectum | 10.6 | Significant increase in colonic IFN-γ levels in PI-IBS patients compared to IBS or controls (*p*<0.05 for both).Significant decrease in colonic IL-10 expression & protein levels in PI-IBS patients compared to IBS or controls (*p*<0.05).No change in IL-2, IL-4 in PI-IBS or IBS patients or controls (*p*>0.05). |
| IBS | Gao *et al.* | 2013 | Experi & Ther Med | Colon biopsies | Sigmoid | 10.5 | Significant increase in colon levels of IL-1β in patients with IBS compared to controls (*p*<0.05).Significant decrease in colon levels of IL-10 in patients with IBS compared to controls (*p*<0.05).IBS-D patients with anxiety & depression had significantly higher levels of IL-1β in colon when compared to IBS-D patients without anxiety & depression (*p*<0.05).IBS-D patients with anxiety & depression had significantly lower levels of IL-10 in colon when compared to IBS-D patients without anxiety & depression (*p*<0.05). |
| IBS | Macsharry *et al.* | 2008 | Scand J Gastroenterol | Colon biopsies | Proximal to junction of sigmoid colon & rectum | 15.5 | Significant downregulation of mRNA expression of IL-1β, CCL20 and transcription factor EGR1 in biopsies from IBS patients when compared to controls (*p*<0.05 for all). Significant decrease in IL-10 and TGF-β expression in mucosa of IBS patients compared to controls (*p*<0.05 for both).No difference in expression of IL-12 between patients & controls. |
| IBS | Ohman *et al.* | 2005 | Clin Gastroenterol & Hepatol | Colon biopsies.  | Ascending, sigmoid. | 12.5 | No significant change in IFN-γ levels in ascending colon between IBS patients & controls. |

Table S5: Studies presenting circulating cell population findings.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **FGID** | **Study** | **Year** | **Journal** | **Blood samples** | **Quality score (/20)** | **Circulating cell population findings** |
| FD & IBS | Kindt *et al.* | 2009 | Neurogastrol Motil | Whole blood. | 13 |  Increased no. of CD3+CD45RA+ & CD3+CD45RO+ cells in patient blood (*p*<0.01) but no other differences between cell populations. |
| FD & IBS (FD focused) | Liebregts *et al.* | 2011 | Am J Gastroenterol | PBMCs.  | 14 | No change in percentage of CD4+ T cells in PBMCs of FD or IBS patients, & controls.Increased numbers of CD4+α4+β7+CCR9+ T lymphocytes in FD patients compared to IBS & healthy controls (*p*<0.001 for both).Increased CD4+α4+β7+CCR9+ T lymphocyte numbers correlated with higher pain intensity, cramps, nausea & vomiting.No association with T cells & fullness or early satiety symptoms. |
| IBS | Holmen *et al.* | 2007 | Neurogastrol Motil | PBMCs.  | 11 | No difference in the number of Treg cells in blood between IBS patients & controls.No change in Treg number cells in blood between IBS subtypes. |
| IBS | Ohman *et al.* | 2009 | Am J Gastroenterol | PBMCs. | 11.5 | No difference in T cell phenotype between IBS subtypes or numbers of CD4+ and CD3+ T cells between IBS patients & controls.Increased numbers of CD4+ & CD8+ cells expressing CD69+ in IBS patients compared to controls (*p*<0.05).Increased CD4+ and CD8+ T cells with HLA-DR+ and integrin β7 in IBS patients compared to controls (p<0.05).No difference in T cells expression of CD25 between IBS patients & controls.No correlation between activated T cell number & IBS symptom severity. |
| IBS | Ohman *et al.*  | 2009 | Neurogastrol Motil | PBMCs. | 12.5 | B cells from IBS patients had increased expression of surface IgG compared to controls (*p*<0.05).Higher expression of CD80 & CD86 on B cells from patients with IBS compared to controls (p<0.05).No difference in CD40 expression on B cells from patients with IBS compared to controls. |
| IBS | Ohman *et al.* | 2012 | Eur J Gastroenterol Hepatol | PBMCs. | 14 | No difference in total number of monocytes (CD11c+CD14+) isolated from PBMC between patients & controls.  |

*Table S6: Studies presenting lamina propria cell population findings.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **FGID** | **Study**  | **Year** | **Journal** | **GIT samples analysed** | **Biopsy sample site** | **Quality score (/20)** | **Lamina propria cell population findings** |
| FD | Futagami *et al.* | 2010 | Am J Gastroenterol | Gastric & small intestinal biopsies.  | Gastric: body & corpus Small intestine: D1 & D2 | 13 | Increase in number of CD68+ve cells in PI-FD compared to EPS, PDS & controls (*p*<0.05 for all).No difference in numbers of CD3+ve, Vdelta1 T cells, or CCR2+ve cells between PI-FD, EPS, or PDS, however CCR2+ve cell numbers were increased in PI-FD when compared to volunteers (*p*=0.009). |
| FD | Gargala *et al.* | 2007 | World J Gastroenterol | Gastric & small intestinal biopsies | Gastric: antrum Small intestine: D1 &d2 | 12 | Significant increase in IEL count in *H. pylori* +ve FD patients & healthy controls by flow cytometry (*p*<0.05 for both).Increased number of CD8+CD3+ IEL cells in *H.* *pylori* +ve FD patients compared to controls (*p*=0.01*).* |
| IBS | Akbar *et al.* | 2008 | Gut | Colon biopsies. | Rectosigmoid junction | 11.5 | Greater percentage of CD3+ cells in IBS patient group compared to controls (*p*=0.03).No significant difference in number of CD4+ cells between IBS group or controls (*p*=0.29). |
| IBS | Chadwick *et al.* | 2002 | Gastroenterology | Colon biopsies. | Ascending, transverse, descending & rectum | 13 | Increased numbers of IELs, lamina propria CD3+ cells & lamina propria CD25+ cells seen in IBS patients compared to controls (*p*<0.05 for all).No significant correlation between age & lamina propria CD3+, CD8+ or CD25+ cell number (*p*<0.01 for all).Greater numbers of IELs & lamina propria CD3+ cells in IBS-D & IBS-M compared to IBS-C (*p*<0.05 for both). |
| IBS | Forshammar *et al.* | 2008 | Scand J Gastroenterol | Colon biopsies. | Ascending & sigmoid | 13.5 | Significant decrease in the number of IgA+ B cells in ascending colon of IBS patients compared to controls (*p*=0.039).Decreased number of IgA+ B cells in ascending colon compared to sigmoid colon in IBS patients (*p*=0.04), this was not seen in healthy controls. No change in number of IgM+, IgE+ or IgG+ B cells between IBS patients & controls (*p*>0.05 for all)*.* |
| IBS | Guilarte *et al.* | 2007 | Gut | Small intestinal biopsies.  | Jejunum.  | 12.5 | Slight increase in the number of CD3+ IEL cells in IBS-D patients when compared to controls (*p*=0.006). |
| IBS | Holmen *et al.* | 2007 | Neurogastrol Motil | Colon biopsies. | Ascending, sigmoid. | 11 | No difference in the number of Treg cells in investigated colon segments between IBS patients & controls.No change in Treg number cells in colon between IBS subtypes.No change in FOXP3 mRNA expression levels between IBS patients 7 controls. |
| IBS | Ohman *et al.* | 2009 | Am J Gastroenterol | Colon biopsies.  | Ascending & sigmoid | 11.5 | No difference in T cell phenotype between IBS subtypes or numbers of CD4+ and CD3+ T cells between IBS patients & controls.Increased numbers of CD4+ & CD8+ cells expressing CD69+ in IBS patients compared to controls (*p*<0.05).Increased CD4+ and CD8+ T cells with HLA-DR+ and integrin β7 in IBS patients compared to controls (p<0.05).No difference in T cells expression of CD25 between IBS patients & controls.No correlation between activated T cell number & IBS symptom severity. |
| IBS | Ohman *et al.* | 2005 | Clin Gastroenterol & Hepatol | Colon biopsies.  | Ascending, sigmoid. | 12.5 | Increased frequency of CD4+ & CD8+ β7+ T lymphocytes in IBS patients compared to controls (*p*=0.02 for both).Increased numbers of both CD4+ & CD8+ lymphocytes in LP of the ascending colon of IBS patients compared to controls (*p*<0.01 for both).No change in number of β7+ T lymphocytes between IBS patients & controls. |
| IBS | Sundin *et al.* | 2014 | Scand J Gastroenterol | Colon biopsies. | Sigmoid | 15 | No change in number of CD3+ IELs or CD3+ lymphocytes between PI-IBS patients & controls.PI-IBS patients had a higher proportion of CD4+CD8+ T cells in LP compared to controls (*p*<0.05).Decreased proportion of CD19+ B cells in LP of PI-IBS patients compared to controls (*p*<0.05).Higher proportion of activated (CD45RO+) LP lymphocytes in PI-IBS patients compared to controls (*p*<0.05). |
| IBS | Cremon *et al.* | 2009 | Am J Gastroenterol | Colon biopsies.  | Descending colon.  | 16 | Significant increase in area of lamina propria occupied by immune cells in IBS patients compared to controls (*p*<0.001).Increases in the area occupied by CD4+, CD3+ and CD8+ T cells in IBS patients compared to controls (*p*<0.05).No change in area occupied by CD79+ B cells between IBS patients or controls. |
| IBS | Braak *et al.* | 2012 | Am J Gastroenterol | Colon biopsies.  | Descending & ascending colon. | 14 | Significant decrease in numbers of CD68+ macrophages in descending colon of IBS patients compared to controls (*p*<0.05).No change in CD3+ T cells, regulatory T cells (FOXP3+), CD163+ macrophages between IBS patients and controls. Significant decrease in CD8+ T cell numbers in IBS females but not IBS males or controls (*p*<0.05). |

Table S7: Studies presenting inflammatory & histological findings.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **FGID** | **Study**  | **Year** | **Journal** | **Histological samples analysed** | **Biopsy sample site** | **Quality score (/20)** | **Inflammatory findings** |
| General population | Walker *et al.* | 2009 | Aliment Pharmacol Ther | Oesophageal, gastric & small intestinal biopsies.  | Gastric: body & antrum.Small intestine: D1 & D2 | 15 | Found no significant association between FGID status & IEL no. in D2 segment of small intestine (*p*=0.32) however a significant association between FGID status & D1 IEL number was observed (*p*=0.016).Significant associations between mast cell number & FGID status in D1 and D2 segments (*p*<0.001).Significant duodenal eosinophilia in D2 segment of FD patients compared to controls, IBS-C& IBS-D (*p*<0.001).Significant overall association between FGID status & eosinophil counts in D1 & D2 (*p*<0.001). |
| FD | Wang *et al.* | 2015 | Ann Diag Path | Small intestinal biopsies | Small intestine: D1 & D2 | 16 | No significant difference in eosinophil counts at D1, however both total number (*p*=0.0013) and number of degranulated cells (*p*=0.0008) increased in D2 biopsies of patients compared to controls. Increased number of mast cells at both D1 & D2 (*p*<0.001 for both), with a significant increase in number of degranulated cells in both D1 & D2 for FD patients (*p*<0.001 for both). |
| FD | Du *et al.* | 2016 | Nat Scientific Reports | Gastric & small intestinal biopsies. | Gastric: body & antrum.Small intestine: D1 & D2 | 16 | No significant difference in eosinophil numbers between patients & controls in the antrum (*p*=0.387), body (*p*=0.223), D1 (*p*=0.86) or D2 (*p*=0.656).Degranulation of eosinophils observed in a greater number of patients than controls in D2 (*p*=0.003) but not in D1 (*p*=0.084).No association between EPS or PDS & gastroduodenal eosinophil counts (*p>*0.05 for all).No association between *H. pylori* infection & duodenal eosinophil number (*p*>0.05). No difference in mast cell numbers between patients & controls in either D1 or D2 (*p*>0.05 for both).  |
| FD | Futagami *et al.* | 2010 | Am J Gastroenterol | Gastric & small intestinal biopsies.  | Gastric: body & corpus Small intestine: D1 & D2 | 13 | Significant relationship found between epigastric burning & degree of histological duodenitis in patients with PI-FD compared to controls (*p*=0.03).Increased numbers of CCR2+ macrophages & eosinophils in PI-FD patients compared to controls. |
| FD | Gargala *et al.* | 2007 | World J Gastroenterol | Gastric & small intestinal biopsies | Gastric: antrum Small intestine: D1 &d2 | 12 | No difference in IEL number in duodenum for *H. pylori* -ve FD patients & healthy controls microscopically (*p*>0.05). Significant increase in IEL count in *H. pylori* +ve FD patients & healthy controls microscopically & by flow cytometry (*p*<0.05 for both). |
| FD | Talley *et al.* | 2007 | Clin Gastroenterol & Hepatol | Oesophageal, gastric & small intestinal biopsies.  | Gastric: body & antrum. Small intestine: D1 & D2 | 15.5 | Significant increases in duodenal eosinophil numbers in patients with FD compared to control. (*p*<0.05).No significant differences in gastric eosinophil counts between FD patients & controls.No association between *H. pylori* infection and duodenal eosinophilia (*p=0.4*)Significant observation of duodenal eosinophil clustering in FD patients compared to controls (*p*=0.003).Duodenal eosinophil degranulation was not significantly increased in FD patients compared to controls where it was not observed (*p*=0.1). |
| FD | Hall *et al.* | 2003 | Clin Gastroenterol & Hepatol | Gastric biopsies. | Gastric: antrum & corpus | 9 | Significant increase in mast cell number between *H. pylori* -ve FD patients & normal controls in both the antrum & corpus (*p*<0.001 for both) independent of inflammation.Significant increase in mast cell number in the antrum of patients with *H. pylori* +ve FD compared to asymptomatic patients (*p*<0.03).No association between mast cell number & symptoms or symptom subgroups. |
| FD | Vanheel *et al.* | 2014 | Gut | Small intestinal biopsies.  | Small intestine: D2 | 18 | Increased numbers of eosinophils & mast cells in FD patients compared to controls (*p*<0.05 for both) but no correlation between the number of mast cells & number of eosinophils.No significant difference in IEL number between patients & controls (*p*=0.9). |
| IBS | Ahn *et al.* | 2014 | Dig Dis Sci | Colon biopsies.  | Ascending, transverse, descending, sigmoid & rectum | 14 | Higher mast cell, IEL and LP lymphocyte counts in colon of IBS-D patients compared to controls (*p*<0.001 for all).No correlation between mean mast cell, IEL, LP lymphocyte counts in entire colon & either QoL questionnaire. |
| IBS | Barbara *et al.* | 2004 | Gastroenterology | Colon biopsies. | Proximal distal | 11.5 | Significant increase in tryptase+ mast cells in patients with IBS compared to controls (*p*<0.05). No difference between IBS-D and IBS-C subgroups (*p*=0.096).Increased incidence of mast cell degranulation in IBS patients compared to controls (*p*=0.026).Significant increase in histamine release in IBS patients compared to controls (*p*=0.015).Significant correlation between proximity of mast cells to nerves, & the severity & frequency of abdominal pain (*p*<0.001 for both). |
| IBS | Chang *et al.* | 2011 | Am J Gastroenterol | Colon biopsies.  | Sigmoid | 16.5 | No change in lymphocyte numbers, enteroendocine, mast cell or enterochromaffin numbers between IBS & controls, or IBS subtype & controls (*p*=0.059-0.892 for all). |
| IBS | El-Salhy *et al.* | 2010 | Dig Dis Sci | Small intestinal biopsies | pars descendens duodeni | 11 | Decrease in density of secretin-immunoreactive cells in IBS patients compared to controls (*p*<0.01).Decreased CCK cell density in IBS-D patients compared to controls (*p*<0.01). No change in IBS-C patients compared to control.No change in serotonin cell density between patients with IBS & controls. |
| IBS | Foley *et al.* | 2011 | Gastroenterology | Small intestinal biopsies. | Duodenal biopsies, site not specified | 15 | Significant increase in IEL counts in IBS-D patients, that significantly correlated with increased mast cell numbers (*p*<0.05 for both) but not EC cells, compared to controls.No statistical difference in mRNA expression of IL-1, IL-6, IL-10 & IL-13 in IBS patients or controls (*p*>0.05 for all). |
| IBS | Guilarte *et al.* | 2007 | Gut | Small intestinal biopsies.  | Jejunum.  | 12.5 | Slight increase in the number of IEL cells in IBS-D patients when compared to controls (*p*=0.006).Significant increase in CD117+ mast cells in jejunal mucosa of patients with IBS-D compared to controls (*p*<0.001), mostly localised in LP.No difference in serum tryptase measurement between controls & IBS-D.Increase in luminal tryptase in IBS-D patients compared to controls (*p*=0.005).No correlation between mast cell number and tryptase level in lumen.  |
| IBS | Ishimoto *et al.* | 2017 | J Clin Biochem & Nutrition | Colon biopsies. | Ileum, cecum, rectum | 13 | No change in mast cell or eosinophil numbers in any sections between IBS-D patients & controls. |
| IBS | Lee *et al.* | 2008 | J Gastroenterol Hepatol | Colon biopsies. | Rectum | 13.5 | PI-IBS patients had significantly higher counts of EC, mast cells & LP T lymphocytes compared to controls (*p*<0.05).Non-PI IBS patients had significantly increased mast cell numbers (*p*<0.05) but no change in EC or LP T lymphocyte number compared to controls.No correlation between EC, mast cell or LP T lymphocyte number, & anxiety & depression. |
| IBS | O'Sullivan *et al.* | 2000 | Neurogastrol Motil | Colon biopsies. | Caecum, ascending, descending, rectum | 13.5 | Increase in mean volume density of mast cells in caecum of IBS patients compared to controls (*p*<0.05). No difference in mast cell number in ascending, descending colon or rectum between groups.No differences in plasma cell, lymphocyte, eosinophil, neutrophil or macrophage number between IBS patients and controls. |
| IBS | Sohn *et al.* | 2014 | Scand J Gastroenterol | Colon biopsies. | Rectum | 17.5 | IBS-D patients had significantly higher numbers of mast cells in rectal mucosa compared to controls (*p*<0.01).Females with IBS-D had significantly higher mast cell counts than female controls (*p*<0.01), however no statistically different count between male IBS-D patients & male controls. Higher levels of Substance P in IBS-D patients compared to controls (*p*<0.01).Female IBS-D patients had significantly higher levels of VIP compared to female controls (*p*<0.01).No correlation between mast cell counts & symptoms including abdominal pain, bloating & stool consistency. |
| IBS | Vicario *et al.* | 2015 | Gut | Small intestinal biopsies.  | Proximal jejunum | 14 | No significant difference in the number of IELs or LP lymphocytes in IBS-D patient biopsies compared to controls.Higher B cell density in IBS-D patients compared to controls (*p*=0.014).Increase in mucosal plasma cell number in patients with IBS-D compared to controls (*p*=0.026). No difference in number of jejunal mast cells between IBS-D patients & controls. |
| IBS | Wang *et al.* | 2007 | World J Gastroenterol | Colon biopsies. Small intestinal biopsies. | Terminal ileum. Descending duodenum, jejunum. | 12.5 | No significant change in number of EC cells in duodenum, jejunum or ileum of patients with IBS-D or IBS-C. Significant decrease in the 5-HT concentration of IBS-C patients compared to controls (*p*<0.05). No change when IBS-D patients 5-HT levels are compared to controls.Increase in the number of mast cells in the ileum of patients with both IBS-D & IBS-C when compared to controls (*p*<0.05 for both). |
| IBS | Park *et al.* | 2006 | J Gastroenterol Hepatol. | Colon biopsies.  | Terminal ileum, ascending colon & rectum.  | 14 | Significant increase in numbers of mast cells in the ileum, ascending colon and rectum of IBS-D patients compared to controls.No change in proportions of plasma cells, neutrophils, lymphocytes or eosinophils between IBS-D patients and controls in the ileum, ascending colon or rectum. |
| IBS | El-Salhy *et al.* | 2013 | Mol Med Rep | Colon biopsies. | Rectum | 12 | No change in number of IELs between controls and IBS patients. No change in number of lamina propria lymphocytes between controls and IBS patients.No change in numbers of mucosal mast cell density in the rectum of IBS patients compared to controls. |
| IBS | Lee *et al.* | 2013 | J Neurogastroenterol Motil | Colon biopsies. | Rectum | 12 | Significant increase in tryptase activity in rectal biopsies from IBS-D patients compared to controls (*p*<0.05).No significant change in rectal mast cell count between IBS-D patients and controls.Significant correlation between mast cell number and intestinal permeability (*p*<0.05) but no relationship between permeability and tryptase activity. |
| General population | Walker *et al.* | 2014 | Human Pathology | Colon biopsies. | Distal ileum, caecum, transverse colon, sigmoid colon & rectum. | 15 | Positive association between IBS and spirochetosis, particularly in the non-constipation type IBS. |
| IBS | Cremon *et al.* | 2009 | Am J Gastroenterol | Colon biopsies.  | Descending colon.  | 16 | Increases in area occupied by mucosal mast cells in IBS patients compared to controls (*p*<0.05). |
| IBS | Braak *et al.* | 2012 | Am J Gastroenterol | Colon biopsies.  | Descending & ascending colon. | 14 | Significant decrease in numbers of mast cells (CD117+) in descending colon of IBS patients compared to controls (*p*<0.05). |