Introduction

Cellular senescence, permanent cell cycle arrest, is a hallmark of ageing (López-Otín et al., 2013). Higher numbers of senescent cells have been reported in aged tissue from not only mice (Wang et al., 2009; Yousefzadeh et al., 2020) but also humans (Tuttle et al., 2020). Furthermore, higher numbers of senescent cells are also reported in age-related disease pathologies including (but not limited to); atherosclerosis, dementia, diabetes and kidney disease (Childs et al., 2017). The detrimental impact the accumulation of senescent cells has on tissue homeostasis has been demonstrated via the removal of senescent cells. Removing senescent cells alleviates symptoms of age-related diseases and physical dysfunction in both animals (Farr et al., 2017; Kim et al., 2019; Pathak et al., 2018; Xu et al., 2018; Yousefzadeh et al., 2018) and humans (Hickson et al., 2019; Justice et al., 2019).

Investigations of cell senescence and age-related diseases have been largely dictated by the availability of human tissue samples. Furthermore, a wide range of markers have been used to detect senescent cells. The proposed gold standard for the detection of senescent cells is to use two or more different markers known to be expressed by senescent cells (Gorgoulis et al., 2019). These markers include; the cell-cycle regulators (p16, p21, p53), proliferation markers (Ki67), DNA damage markers (γH2Ax, Bmi1), SA-beta-gal, Lamin B1, chromatin markers (SAHF, SADS) and/or the senescence associated secretory phenotype (SASP) (Gorgoulis et al., 2019). However, determining which markers to use to detect senescence is largely at the discretion of the researchers conducting the experiment. This has resulted in different sets of markers used to detect senescent cells in similar pathologies producing
conflicting results (Akram et al., 2014; Okuda et al., 2019). Whether these results merely represent a difference in number of senescent cells or demonstrate that specific age-related pathologies have specific senescent phenotypes is unknown.

The role of senescent cells in age-related diseases is an extensively reviewed topic (Baker and Petersen, 2018; Campisi et al., 2011; Childs et al., 2015; Kirkland, 2017; Sturmlechner et al., 2017) however, most reviews are narrative in nature and do not focus on evidence solely available in humans. As such to provide an overview of the current state of the literature a systematic review focused on senescent cells within age-related pathologies in humans was conducted.

2. Methods

2.1. Selection of studies

A systematic search of the literature was performed in PubMed, EMBASE and Web of Science from date of inception to 29th of September 2019 using the terms “senescence”, “tissue/biopsy/histology”, different “organ/tissue types” and different “markers of cellular senescence” (see Supplementary Information 1). Title and abstract screening was performed independently by two reviewers (M.W., C.T., and/or S.L.) to exclude irrelevant articles. All conflicts were resolved by a third reviewer (A.M.). Exclusion criteria were defined as: i) studies with only animal populations; ii) in vitro studies; iii) reviews and conference abstracts; iv) method/theory papers (without primary data sources); v) articles investigating the association between senescence and age (no pathology reported); vi) articles written in a language other than English. Full text of the remaining articles was screened by two reviewers (M.W., C.T., and/or S.L.) using the aforementioned exclusion criteria and the following additional criteria: vii) marker to detect senescent cells not reported; viii) no comparison of the expression of senescent marker between disease pathology and control pathology; ix) cancer pathology x) telomere length as the only reported marker of senescence. Telomere length was excluded as a marker of senescence as telomere length has not been established (reviewed in (Cristofalo et al., 2004; Sharpless and Sherr, 2015)). Any disagreement between reviewers was arbitrated by an additional reviewer (A.M.).

2.2. Data extraction

Data extraction was performed by two reviewers (S.L., J.W.) and conflicts were resolved by a third reviewer (C.T.). Using a standardized data extraction form, the following data was extracted: age-related disease; type and origin of tissue; the sample size, chronological age and sex of the participants; the marker(s) used to determine senescence and the technique used; and all data analysis comparing the senescence markers between age-related pathology and control tissue. Senescence markers were further categorized into ‘type of markers’ as determined by the markers role within the cell cycle. For analysis purposes, the reported direction of markers known to be negatively associated with senescence e.g. proliferation markers, were reversed. If an article reported on senescence of more than one tissue type within a population, this information was extracted as separate associations in order to establish if the association between senescence and age-related diseases was consistent across and within tissues samples.

2.3. Statistical analysis

A meta-analysis combining the extracted data to determine overall senescence within a disease state for each organ system could not be performed due to heterogeneity of markers used and statistical presentation of data. Associations of senescence with age-related diseases compared to controls were visualized using a heat map based on reported p-values. Based on the p-values the results of the associations were grouped into three categories: p ≤ 0.05; expression of senescence markers higher in the pathology samples compared to controls, p ≥ 0.051 inconclusive, and p ≤ 0.05 expression of senescence marker is higher in controls compared to pathology samples.

3. Results

3.1. Selection of included studies

The literature search retrieved 12,590 articles (Fig. 1). After duplicates were removed title and abstract screening occurred for 7685 articles and 954 full text articles were assessed for eligibility. Overall, 103 articles were included in this systematic review.

3.2. Characteristics of included studies

Table S1 provides a comprehensive overview of all included articles. The number of included participants was reported in 98/103 articles and the total number was 10,469 participants. One of the articles (Skowronska-Krawczyk et al., 2015) contributed 5459/10,469 participants to this overall total. The sex of the populations studied was provided in 45/103 articles and 2188/3977 (55%) of the population were male (Table 1). The expression of senescence markers in diseased participants has been examined in nine different organ systems and 27 different age-related diseases. Diseases of the heart (27/103), liver (20/103) and respiratory systems (18/103) were most investigated. The majority (62/103) of articles utilized more than one marker to detect senescent cells within tissue samples. Overall, 27 different markers of senescence have been used to determine cellular senescence and the cell-cycle regulators, p16 (23/27 diseases), p21 (19/27 diseases) and p53 (18/27 diseases) were the most frequently used markers to detect senescence (Fig. 2).

Table S2 provides a comprehensive overview of outcome data for all included articles stratified by organ systems.

3.2.1. Brain

Overall, 11/103 articles focused on diseases of the brain with Alzheimer’s Disease as the most studied pathology (4/11). All articles (Al-Mashhadi et al., 2015; Bhat et al., 2012; Chinta et al., 2018; Garwood et al., 2014; Musi et al., 2018; Nicaise et al., 2019; Schas et al., 2019; Shankbag et al., 2019; Turnquist et al., 2016; Vazquez-Vilaseñor et al., 2019), except one (Sorensen et al., 2016), utilized brain tissue to determine the association between senescence and disease pathology. Higher levels of markers of senescence were observed in all studies irrespective of the disease state (Fig. 2). Some markers of senescence (p53, Sañ-Gal, γH2Ax) were not reported to be significantly higher within some disease pathologies (Alzheimer’s Disease, and motor neuron disease). In addition, some markers were expressed differently within tissues samples (Table S2). For example p16 and γH2Ax in the glia cells of the medulla cortex were significantly lower in motor neuron patients compared to controls whereas both p16 and γH2Ax were significantly higher in the glia cells from the frontal cortex in the motor neuron patients compared to controls (Vazquez-Vilaseñor et al., 2019).

3.2.2. Eye

Overall, 6/103 articles focused on diseases of the eye including endothelial corneal dystrophy (2/6) (Matthaei et al., 2013, 2012), glaucoma (2/6) (Litton et al., 2005; Skowronska-Krawczyk et al., 2015), cataract (1/6) (Yan et al., 2019) and retinal microaneurysms (1/6) (Lopez-Luppo et al., 2017). All articles utilized eye tissue to determine an association between senescence and disease pathology, and one glaucoma study (Skowronska-Krawczyk et al., 2015) also used blood samples. Higher levels of the markers of senescence were observed in all studies irrespective of the disease state (Fig. 2). Some markers of
senescence (p16, p21, Saβ-Gal) were not reported to be significantly higher compared to controls for some diseases (Table S2). In endothelial corneal dystrophy populations, higher levels of p21 were significantly associated with the disease, but not with p16 (Matthaei et al., 2013).

3.2.3. Endocrine
Overall, 12/103 articles focused on diseases of the endocrine system with type 2 diabetes (5/12) (Fomison-Nurse et al., 2018; Gustafson et al., 2019; Minamino et al., 2009; Prattichizzo et al., 2018; Soundararajan et al., 2019) and diabetic nephropathy (5/12) (Chen et al., 2017; Guo et al., 2014; Liu et al., 2012; Tsai et al., 2018; Verzola et al., 2008) as the most studied pathology. All articles investigating Type 2 Diabetes used adipose tissue, peripheral blood mononuclear cells (PBMC) and artery tissue, whereas diabetic nephropathy was studied utilizing kidney tissue. Higher levels of expression of markers of senescence were observed in all studies irrespective of the disease state, except for one study utilizing blood monocytes to determine if p16 and the SASP markers IL-6 and IL-8 were higher in diabetic participants compared to control (Prattichizzo et al., 2018). SASP markers were most often studied in endocrine disease compared to any other disease pathology (Fig. 2). The expression of p53 and p21 did not differ in macrophages between study groups (Akram et al., 2014). While the expression of the senescence markers was higher in the disease cohort compared to the control groups, the expression of these markers varied depending upon the cell type investigated.

3.2.4. Respiratory
Overall, 18/103 articles focused on diseases of the respiratory system with chronic obstructive pulmonary disease (COPD) (13/18) being the most studied pathology (Amselem et al., 2011; Aoshiba et al., 2012; Gu et al., 2017; Habiel et al., 2017; Hashimoto et al., 2016; Houssaini et al., 2017; Lakhdar et al., 2018; Nouredine et al., 2011; Okuda et al., 2019; Saito et al., 2006, 2010; Zhou et al., 2011). Most articles (17/18) utilized lung tissue to determine cellular senescence and the cell type utilized varied and included endothelial cells (Amselem et al., 2011; Aoshiba et al., 2012; Houssaini et al., 2017; Tsuji et al., 2006), Type I Cells (Aoshiba et al., 2012), Type II cells (Aoshiba et al., 2012; Disayabutr et al., 2016; Lehmann et al., 2017; Tsuji et al., 2006, 2010), Clara cells (Zhou et al., 2011), SP-C cells (Tsuji et al., 2010), macrophages and lymphocytes (Akrum et al., 2014). The comparison of the expression of these markers within the airways were consistently compared across three groups; non-smokers, smokers and diseased participants (10/18) (Aoshiba et al., 2012; Disayabutr et al., 2016; Hashimoto et al., 2016; Okuda et al., 2019; Saito et al., 2019; Tsuji et al., 2006, 2010; Zhou et al., 2011). Higher levels of expression of markers of senescence were observed in all studies irrespective of the disease state (Fig. 2). The expression of p53 and p21 did not differ in macrophages between study groups (Akrum et al., 2014). While the expression of the senescence markers was higher in the disease cohort compared to the control groups, the expression of these markers varied depending upon the cell type investigated.

3.2.5. Heart
Overall, 27/103 articles focused on diseases of the heart with atherosclerosis (8/27) (Abbasi et al., 2017; Eo et al., 2011; Gardner et al., 2015; Lv et al., 2017; Matsumoto et al., 2009; Matthews et al., 2006; Minamino et al., 2002; Vasil et al., 2001) and coronary artery disease (7/27) (Chan et al., 2017; Cui et al., 2018; Jia et al., 2017; Lewins-McDougall et al., 2019; Marfell et al., 2012; Piegarre et al., 2013; Yuan et al., 2010) the most studied pathology. Most articles (24/27) utilized tissue from the heart to determine senescence, but the type of tissue varied between studies and included: coronary artery, mammary artery, aorta, femoral artery, aortic valve right atrium and the myocardium. Higher expression of senescence markers was observed in all studies irrespective of the disease state (Fig. 2). Notably most atherosclerosis studies (6/8) did not quantify the level of senescence within tissue.
### Table 1

Overview of included articles, stratified by organ system (bold) and tissue used.

| Disease (Specimen) | Articles | Subject | Sex, M | Senescence Marker |
|--------------------|----------|---------|--------|-------------------|
| **Brain** | | | | |
| Cognitive Impairment | 2 | 224 | 201 | p16, p53, γH2Aγ, SA-γgal |
| **Brain tissue** | 1 | 43 | 20 | p16 |
| Salivary gland tissue | 1 | 181 | 181 | p16, p53, γH2Aγ, SA-γgal |
| Alzheimer's Disease, Brain tissue | 4 | 168 | 39/67 | p16, p21, p53, γH2Aγ, SA-γgal, SARC (IL-6), SAβgal, SSBP1 (IL-6), SSBP2 |
| **Dementia, Brain tissue** | 2 | 16 | NR | p16, γH2Aγ, SASP (IL-6, IL-1α, IL-8, MMP3) |
| **PSP, Brain tissue** | 1 | 24 | 15 | p16 |
| **MS, Brain tissue** | 1 | 12 | 7 | p16 |
| **Eye** | | | | |
| Endothelial Corneal Dystrophy, Eye tissue | 2 | 75 | 25 | p16, p21 |
| Glaucoma | 2 | 4236 | NR | p16, SA-γgal |
| Blood | 1 | 4224 | NR | p16, SA-γgal |
| Eye tissue | 1 | 12 | NR | SA-γgal |
| Cataract, Lens capsules | 1 | 149 | NR | p21, p53, Laminin, LMO4, SA-γgal, SARC (TGFB-γ) |
| **Retinal Microaneurysm, Eye tissue** | 1 | 34 | 16 | p16, p21, p53, SA-γgal |
| **Endocrine** | | | | |
| CVD, Kidney | 1 | 178 | NR | p16 |
| Obese, Adipose tissue | 1 | 74 | NR | p53 |
| **Type 2 Diabetes** | 5 | 143 | NR | p16, p21, p53, SA-γgal SASP (IL-6, IL-8), TNFα, TGFB-γ |
| Adipose tissue | 2 | 25 | NR | p16, p21, p53, SA-γgal SASP (TGFB-γ) |
| Blood | 2 | 83 | 31/60 | p16, p21, p53, SA-γgal SASP (IL-6, IL-8), TNFα |
| Atrial Appendage | 1 | 33 | 26 | p53 |
| Diabetic Nephropathy, Kidney tissue | 5 | 375 | 189 | p16, p21, p53, SA-γgal SASP (IL-6, IL-8, MCP-1) |
| **Respiratory** | | | | |
| Chronic Obstructive Pulmonary Disease | 13 | 458 | 275/400, NR-58 | p16, p21, p53, SA-γgal γH2Aγ, SASP (IL-6, IL-8), Clumperin |
| Lung tissue | 12 | 428 | 251/370 | p16, p21, p53, SA-γgal γH2Aγ, SASP (IL-6, IL-8), Clumperin |
| **Vastus lateralis** | 1 | 30 | 24 | p16, p21, p53, SA-γgal γH2Aγ |
| **Idiopathic Pulmonary Fibrosis, Lung tissue** | 5 | 148 | 64/100 | p16, p21, p53, SA-γgal γH2Aγ |
| Heart Hypertension | 2 | 97 | 52 | p16, p53 bound to p21, γH2Aγ, SASP (MCP1) |
| **Artery (skeletal)** | 1 | 55 | 34 | p53 bound to p21, γH2Aγ |
| Urine Vesicles | 1 | 42 | 18 | p16, SASP (MCP1) |
| Atherosclerosis | 9 | 186 | 49/80, NR-106 | p16, p21, p53, SA-γgal γH2Aγ |
| Aorta | 3 | 71 | NR | p16, p21, SA-γgal γH2Aγ |

### Table 1 (continued)

| Disease (Specimen) | Articles | Subject | Sex, M | Senescence Marker |
|--------------------|----------|---------|--------|-------------------|
| Coronary artery | 3 | 77 | 38/32, NR-45 | p53, SA-γgal |
| Internal mammary artery | 2 | 20 | NR | p16, p53, SA-γgal |
| Femoral artery | 1 | 18 | 11 | SA-γgal |
| Aortic Valve Stenosis, Aortic tissue | 1 | 33 | 17 | SA-γgal |
| Aneurysm, Aortic tissue | 5 | 135 | 80/110, NR-25 | p16, p19, p21, p53, SA-γgal γH2Aγ, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Coronary Artery Disease | 6 | 237 | 79/148 | p16, p21, p53, Acetyl-p53, γH2Aγ, SARC (IL-6, IL-8), SAβgal |
| Heart Tissue | 4 | 102 | 5/13 | p16, p21, p53, SAβgal |
| Blood monocytes | 1 | 60 | 31 | Acetyl-p53 |
| Myocardium | 1 | 75 | 43 | p16, Ki67 |
| Heart Failure | 4 | 171 | 129 | p14, p16, p21, p27, p53, phos-p53, acetyl-p53, Bmi1, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Myocardium | 3 | 108 | 77 | p14, p16, p21, p27, p53, phos-p53, acetyl-p53, Bmi1, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Cardiomyocytes | 1 | 63 | 52 | SARC (IL-6, IL-8), SAβgal |
| Liver | | | | |
| Non-Alcoholic Fatty Liver Disease, Liver tissue | 3 | 451 | 243, NR-74 | p16, p21 |
| Alcohol Related Liver Disease, Liver tissue | 1 | 136 | 73, NR-17 | p21 |
| Infection Hepatitis | 5 | 437 | NR | 270/422, NR-15 | p16, p21, p27, p53, Acetyl-p53, γH2Aγ, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Liver tissue | 4 | 376 | NR | p16, p21, p27, p53, Acetyl-p53, γH2Aγ, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Liver CD3 cells | 1 | 61 | 34 | p16, p21, p27, p53, Acetyl-p53, γH2Aγ, SARC (IL-6, IL-8), SARC (IL-6, IL-1α) |
| Liver Cirrhosis, Liver tissue | 10 | 581 | 10/16, NR-65 | p16, p21, p53, SA-γgal γH2Aγ, Ki67 |
| Hepatic Encephalopathy, Brain tissue | 1 | 19 | 12 | p21, p53 |
| Kidney | | | | |
| Chronic Kidney Disease | 6 | 314 | 176 | p16, p53, γH2Aγ, SA-γgal SASP (IL-6, IL-8) |
| Kidney tissue | 3 | 118 | 69 | p16, γH2Aγ, SASP (IL-6, IL-8) |
| PBMC | 2 | 135 | 65 | p53, γH2Aγ, SASP (IL-6, IL-8) |
| Skeletal muscle artery | 1 | 61 | 42 | p16, SA-γgal, SARC (IL-6, IL-8) |
| Prostate | | | | |
| Prostate Hypertrophy | 1 | 55 | 55 | p21, mtP53 |
| Skeletal | | | | |
| Disk Degeneration | 1 | 50 | 26 | p53, SA-γgal |
| Lumbar Disks | 1 | 20 | 8 | p53 |
| Chronic Leg Ulcer | 1 | 10 | NR | γH2Aγ |
| Wound Tissue | 1 | 10 | NR | γH2Aγ |

**Abbreviations**: CVD – Cardiovascular disease, PBMC – Peripheral Blood Mononuclear Cells, PSP – Progressive Supranuclear Palsy, MND – Motor Neurone Disease, MS – Multiple Sclerosis, NR – Not reported.
samples and SA-β-gal was the preferred marker to detect senescence (6/8).

3.2.6. Liver
Overall, 20/103 articles focused on diseases of the liver with liver cirrhosis (9/20) (Gutierrez-Reyes et al., 2010; Sasaki et al., 2015, 2005; Sasaki et al., 2006, 2010a; Sasaki et al., 2008, 2010b, c; Wiemann et al., 2002) the most studied pathology. Most articles (19/20) (Aravinthan et al., 2013, 2012; Chiba et al., 2011; Gutierrez-Reyes et al., 2010; Ikeda et al., 2009; Kim et al., 2009; Nakajima et al., 2010; Sasaki et al., 2015, 2005; Sasaki et al., 2006, 2010a; Sasaki et al., 2008, 2010b, c; Tachtatzis et al., 2015; Wandrer et al., 2018; Wiemann et al., 2002; Yoon et al., 2011) utilized tissue from the liver to determine senescence expression. Higher levels of expression of markers of senescence were observed in all studies irrespective of the disease state. Notably within these articles the expression of senescence was often examined within the small bile ducts and bile ductules within the tissue samples and between groups with increasing severity of disease.

3.2.7. Kidney
Overall, 6/103 articles focused on chronic kidney diseases (Crepin et al., 2018; Melk et al., 2005; Ramirez et al., 2005; Sis et al., 2007; Stenvinkel et al., 2017; Westhoff et al., 2008). Higher levels of expression of markers of senescence were observed in all studies irrespective of the disease state. Notably the expression of senescence differed between different cell types of the kidney (glomeruli, arteries, tubuli), but all cell types were found to have higher levels of senescent markers (except for γH2Axl) in the disease pathology compared to controls.

3.2.8. Prostate, skeletal and venous ulcer
Overall, 1/103 articles focused on diseases of the prostate (Torrealba et al., 2018), 1/103 articles focused on disc degeneration (Gruber et al., 2011) and 1/103 articles investigated senescence in chronic venous leg ulcers (Sindrilaru et al., 2011). Higher levels of expression of markers of senescence were observed in all of the pathology samples compared to controls.

4. Discussion
In this review we have collated all current evidence investigating senescence in age-related diseases within human populations. We demonstrate that higher expression of senescence markers are observed within disease populations compared to the control populations. However, the expression of some senescence markers within a tissue sample maybe dependent upon the disease.

Our findings corroborate previous reviews that demonstrate senescence is associated with age-related diseases. (Baker and Petersen, 2018; Childs et al., 2017, 2018; Kirkland et al., 2017) However, here we also demonstrate that the cell cycle regulators particularly; p16, p21 and p53 are consistently upregulated in diseased human tissue samples. Overall, most studies utilized p16 as a marker to detect senescent cells and reported a higher expression of p16 within a disease state. The findings of the four studies that did not detect higher p16 levels in diseased tissue (Gutierrez-Reyes et al., 2010; Lewis-McDougall et al., 2019; Matthaei et al., 2013; Prattichizzo et al., 2018) were not replicated by other groups investigating similar disease pathologies. On the other hand, p21 and p53 were higher in all disease populations compared to controls except for two studies which reported a significantly lower expression of...
p21 in heart failure patients (Urbanek et al., 2005) and p53 in patients with an aneurysm (Blunder et al., 2018). This analysis suggests that p16 should be included as a marker in any senescence panel in diseased human tissue. It is important to consider that while higher expression of these markers were observed in most pathologies the level of expression varied dependent on the control groups. Specifically, studies that investigated the expression of senescence markers within COPD populations demonstrated higher p16 levels in the COPD population compared to non-smokers, but non-significantly different p16 levels compared to smokers (Aoshiba et al., 2012; Tsuji et al., 2006, 2010; Zhou et al., 2011). On the other hand, the overall difference between p21 expression in COPD patients compared to both non-smokers and smokers seemed to be consistent across studies (Amselem et al., 2011; Gu et al., 2017; Houssaini et al., 2017; Noureddine et al., 2011; Tsuji et al., 2006). This may indicate that environmental factors, such as smoking can influence p16 senescence, whereas higher levels of p21 may be indicative of a disease phenotype in the airways. The first-in-human, open label, pilot senolytic trial utilized an IPF patient cohort and the senolytics dasatinib (D) and quercetin (Q). Dasatinib is a second-generation Src tyrosine kinase inhibitor (STKs) which quercetin selectively interferes with the PI3 K/akt and the p53/p21/serpine pathways (Justice et al., 2018). While the authors of this study do not report significant changes directly related to senescent cells within their cohort, they did find that DQ alleviated physical dysfunction in IPF patients. Current research to detect senescence within human tissue samples has largely utilized the cell cycle regulators, p16, p21 and p53. However, the findings of this review suggests that ambiguity still surrounds whether these senescence markers are upregulated in all disease pathologies. There is a considerable lack of use of cell cycle regulators p19, p27 and Cyclin D to detect senescence levels with human tissue samples. The experimental use of these markers may provide further clarity around senescent phenotypes within human diseases. Furthermore, a combination of cell cycle regulators in addition to a DDR marker and SASP markers may prove to be a more useful and powerful senescence panel rather than a cell-cycle regulator panel.

Overall, most studies utilized two or more markers to determine senescence levels within the studied populations. Interestingly, of the markers utilized to detect senescence the proliferation marker Ki67 reported the most conflicting results (Blunder et al., 2018; Ikeda et al., 2009; Kim et al., 2009; Marfella et al., 2012; Nakajima et al., 2010; Noureddine et al., 2011; Sasaki et al., 2010a) When a cell is senescent it can no longer undergo proliferation, as such Ki67 is expected to be downregulated in senescent cell populations. However, while in some disease pathology downregulation of Ki67 (Kim et al., 2009; Marfella et al., 2012; Nakajima et al., 2010) was observed, other disease pathologies demonstrated higher levels of Ki67 (Blunder et al., 2018; Noureddine et al., 2011; Sasaki et al., 2010a). While the disease pathology differs between each of these studies, the conflicting results are from tissue samples of the heart and liver. A recent single cell study by Miller and colleagues demonstrated that Ki67 is degraded continuously in G1 and G0 phases irrespective of the cause of entry into G0. In their study they tracked Ki67 levels under endogenous control in single cells overtime. Their experiment demonstrated that Ki67 accumulated in the S, G2 and M phases of the cell cycle but in the G0 and G1 phases Ki67 expression was highly heterogeneous and largely dependent upon how long a cell was in the G0 state. (Miller et al., 2018) There are a number of factors that influence Ki67 expression within a cell, such as, like all current markers for senescence in tissue, Ki67 should not be used as the sole marker to detect senescence in tissue samples.

The SASP role in mediating and sustaining the senescence paradigm within organs is a well-established phenomenon. (Campa et al., 2011; van Deursen, 2014) However, most of the studies reported in this review did not use a SASP marker in their senescence panel to detect senescence within tissue samples. It has been hypothesized that the SASP profile of senescent tissue is likely to be unique to specific tissue and the disease phenotype. (Coppe et al., 2010) Whether the diverse array of SASP markers used within each study was chosen based on prior research was unclear. However, given the distinct lack of research into SASP profiles of human tissue sections, this seems unlikely. Recently, Chatsiripsiapai and colleagues performed a transcriptomic analysis across 14 human tissue samples to investigate the relationship between ageing, cancer and cellular senescence. (Chatsiripsiapai et al., 2019) In their analysis they identified different gene profiles of senescent tissues. Such an analysis may be beneficial in defining specific tissue or disease related SASP markers to use in subsequent senescence panels.

The expression of specific senescence markers did differ between cells from the same tissue section. (Amsellem et al., 2011; Aoshiba et al., 2012; Balint et al., 2019; Melk et al., 2005; Noureddine et al., 2011; Sasaki et al., 2015, 2005; Sis et al., 2007; Tsuji et al., 2006; Vazquez-Villaseor et al., 2019; Verzola et al., 2008; Westhoff et al., 2008) This is not unexpected given that the turnover rate of cells is likely to differ within tissues. Furthermore if it is hypothesized that SASP can induce senescence in surrounding cells (Coppe et al., 2010), varying levels of senescence markers would be expected as the surrounding tissue undergoes senescence. Similar to our previous research (Coppe et al., 2020), few studies (Moreno-Navarrete et al., 2015; Skowronska-Krawczyk et al., 2015; Stenvinkel et al., 2017; Tsai et al., 2018) assessed the level of senescent cells across more than one tissue sample from the same individual. As such, whether higher senescent cells are localized to the diseased tissue or whether a rise in senescence levels can be measured systemically is still unknown. The few studies that have examined senescence in more than one tissue from the same individual have shown that senescence levels are higher in a disease state but the levels of senescence differ significantly across tissue samples. (Moreno-Navarrete et al., 2015; Skowronska-Krawczyk et al., 2015; Stenvinkel et al., 2017; Tsai et al., 2018) This review is not without limitations, the search strategy was designed to be broad and inclusive, but specifically focused on human tissues. As such the search strategy did not include terms which would specifically select human studies utilizing peripheral blood samples. The literature, reporting on senescence in human blood samples, ageing and age-related diseases is beyond the scope of this review. Furthermore, studies specifically investigating the role of immunosenescence and age-related diseases have been reviewed elsewhere. (Filop et al., 2016) In addition, while this review focuses on age-related diseases, articles where cancer was the disease of interest, have been excluded. The role of senescence in cancer is complex (Sieben et al., 2018) and the literature surrounding this topic significant and beyond the purview of this review. However, a comprehensive review summarising this research would be of benefit to the field. Due to the complex nature of the extracted data - different diseases, tissue sections, senescent markers, cell types and control groups - a meta-analysis describing the overall level of senescence within disease tissue compared to controls could not be performed. The p-values reported here does not indicate the size or importance of the observed effect and is significantly influenced by the sample size and statistical precision. Furthermore, it is important to note that publication bias towards positive findings can’t be excluded. As such the conclusions of this review should be treated judiciously.

Importantly, this review has also highlighted reporting omissions within this research field. Specifically, the sex of the populations assessed has been omitted in over 50 % of the included studies. Significant research reporting sex differences in ageing and age-related diseases (Blagosklonny, 2010; Caruso et al., 2013; Márquez et al., 2020) exist. How these differences translates to the level of senescence observed in men and women is not well understood (Graves et al., 2006) however, the sex ratio of human tissue samples should be considered when analyzing grouped human data. Similarly, few studies reported on the severity of the disease stage within their study population. Given it is hypothesized that the level of senescence increases with disease severity omitting this variable makes comparisons of senescent levels across and between studies difficult.

This review has confirmed that higher levels of senescence are
observed in age-related diseases compared to controls within humans. Furthermore, the current evidence suggests that p16 is a robust marker for the detection of senescence in human tissue samples. However, more research into whether diseases have a specific senescent phenotype is required.

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Authors contributions
C.T. Literature search and screen, data extraction, collation of results, draft manuscript. S.L. Literature search and screen, data extraction, collation of results, review manuscript. M.W. Original concept, literature search and screen, review manuscript. A.M. Original concept, literature search, review and edit manuscript.

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Appendix A. Supplementary data
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