Pancreatology xxx (2017) 1–9

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Intratumoural leukocyte infiltration is a prognostic indicator among pancreatic cancer patients with type 2 diabetes

Emelie Karnevi [a, b, *], Agata Sasor [c], Katarzyna Said Hilmersson [a], Daniel Ansari [a], Jonas S. Erjefält [d], Ann H. Rosendahl [b, 1], Roland Andersson [a, 1]

[a] Lund University and Skåne University Hospital, Department of Clinical Sciences Lund, Division of Surgery, Lund, Sweden
[b] Lund University and Skåne University Hospital, Department of Clinical Sciences Lund, Division of Oncology and Pathology, Lund, Sweden
[c] Skåne Regional Laboratories, Department of Pathology, Lund, Sweden
[d] Lund University, Department of Experimental Medical Sciences, Division of Respiratory Medicine and Allergology, Lund, Sweden

ARTICLE INFO

Article history:
Received 19 May 2017
Received in revised form
10 September 2017
Accepted 8 November 2017
Available online xxx

Keywords:
Pancreatic cancer
Type 2 diabetes
Macrophages
Neutrophils

ABSTRACT

Background: The life expectancy of pancreatic cancer patients remains minimal. The disease progression may be influenced by type 2 diabetes (T2D) and inflammatory status, although important gaps persist around their joint effects on disease outcome. The aim of this study was to investigate the clinical significance of the tumour immune microenvironment on pancreatic cancer prognosis in relation to T2D status.

Method: Tumour-infiltrating macrophages, neutrophils and eosinophils were studied in primary pancreatic tumours and paired lymph node metastases in relation to patient and tumour characteristics, T2D status and overall survival in a retrospective cohort of patients with resectable pancreatic cancer in Sweden.

Results: Of the 80 included pancreatic cancer patients, 22 (27.2%) had T2D. The diabetic pancreatic cancer patients had significantly higher systemic high white blood cell count than those without diabetes ($P = 0.028$). Macrophage infiltration levels were higher in lymph node metastases compared with primary tumours ($P = 0.040$) among pancreatic cancer patients with diabetes. Type 2 diabetes or intratumoural leukocyte (macrophage, neutrophil or eosinophil) infiltration alone did not significantly influence pancreatic cancer prognosis. However, among cancer patients with T2D high macrophage or neutrophil tumour-infiltration was associated with a significant reduction in overall survival (adjusted hazard ratio [HR] 7.2; 95% CI 1.5–35.0 and HR 5.4; 95% CI 1.1–26.3, respectively).

Conclusion: These results demonstrate associations between T2D and enhanced inflammatory processes with significant implications on survival among pancreatic cancer patients with T2D. Validation in larger independent patient cohorts may identify additional prognostic tools and improved treatment strategies for specific patient subsets.

Introduction

Pancreatic cancer is one of the most devastating types of cancer, with an overall 5-year survival less than 5% and median survival under 6 months [1]. It is currently the fourth leading cause of cancer-related death in Western countries, and expected to rise in the years to come, soon representing the second cause of death in cancer [2]. The poor prognosis is due to late symptoms and difficulties of detection, which together with the aggressive nature of the disease frequently leads to an advance stage at the time of diagnosis. The only potentially curative treatment is surgical resection of the tumour, although this is only possible for 15–20% of patients. In addition, first-line chemotherapy gemcitabine provides limited survival benefits [3,4]. The progressive nature of the disease and scarce treatment options creates an urgent need for early detection, prognostic indicators for improved disease stratifications, treatment prediction in this group of patients as well as and novel therapeutic options in order to improve survival.

Obesity, type 2 diabetes (T2D), chronic inflammation of the

* Corresponding author. Div. Oncology & Pathology, Kampradhuset, Barngatan 4, 22185 Lund, Sweden.
E-mail address: emelie.karnevi@med.lu.se (E. Karnevi).
[1] joint senior authors.

https://doi.org/10.1016/j.pan.2017.11.003
1424-3903/

pancreas (pancreatitis) and smoking have all been linked to increased risk of pancreatic cancer [3,5]. In fact, T2D and obesity are considered among the top modifiable risk factors for pancreatic cancer due to the strong associations between the diseases [6–9]. In addition, T2D is associated with higher risk of cancer-related death among pancreatic cancer patients [10,11].

Both pancreatic cancer and T2D are associated with low-grade systemic inflammation. The local inflammation is believed to be orchestrated by tumour-associated macrophages (TAMs), in a complex interplay with the tumour stroma and cancer cells [12]. Tumour-infiltrating leukocytes, in particular TAMs, and elevated systemic cytokines levels have previously been suggested to influence pancreatic cancer progression [13–15]. High TAM infiltration has been associated with poor survival and TAMs are believed to stimulate invasion, angiogenesis and metastasis [15–17]. Although TAMs have a central role in tumour-associated inflammation, additional leukocyte subsets, such as neutrophils, eosinophils, T-lymphocytes and myeloid-derived suppressor cells (MDSCs) have been shown to promote tumour progression in the local microenvironment [18–21]. They are believed to contribute to suppression of anti-tumour inflammatory responses and stimulate invasion into surrounding tissue. There is an emerging role of pancreatic cancer-related inflammation, which opens up for further investigations of new and additional targets for possible interventions.

Although T2D and inflammation have been shown to influence both the risk and disease outcome, important gaps exist in the understanding of their joint effects on the prognosis of pancreatic cancer patients. The aim of the present study was to investigate the significance of tumour-infiltrating leukocytes (macrophages, neutrophils and eosinophils) on clinical and pathological parameters and pancreatic cancer prognosis in relation to T2D status.

Material and methods

Study population

The present study is based on a retrospective cohort, including patients with resectable pancreatic ductal adenocarcinoma (PDAC) at Skåne University Hospital, Lund, Sweden between February 1995 and May 2013. In total, 288 patients underwent pancreatectomy during the study period. From the pathological examination following surgery, only patients with confirmed PDAC diagnosis were included. To verify the tissue quality and presence of PDAC prior to the study, the paraffin blocks available were re-evaluated by an independent pathologist blinded to the original evaluation. A flow chart of the selection process of patients is shown in Fig. 1. Of the 115 PDAC patients identified from the selection process, tumour tissue was available for 81 patients. Approximately 56% of these patients (45 of 81) had confirmed lymph node metastases, of which tissue was available for 39 patients. Information on patient and clinical characteristics were collected through medical records and pathological reports, including information from blood samples taken at diagnosis. Follow-up information of survival was obtained from the Regional Tumour Registry or the Population Registry. The study was approved by the regional ethics committee in Lund, Sweden (Dnr 2013/410, LUBB 33-10).

Neutrophil and eosinophil infiltration

Hematoxylin and eosin (H&E) staining was performed on 4 μm formalin-fixed paraffin-embedded whole slide tissue sections with reduced exposure to eosin (1 min, 0.1% eosin) to increase contrast in staining of cell types of interest. Neutrophils and eosinophils were counted by a trained pathologist who was blinded to clinical and outcome data in high power fields (HPF: 40× magnification) at 10 representative areas, without signs of necrosis, for each tissue sample.

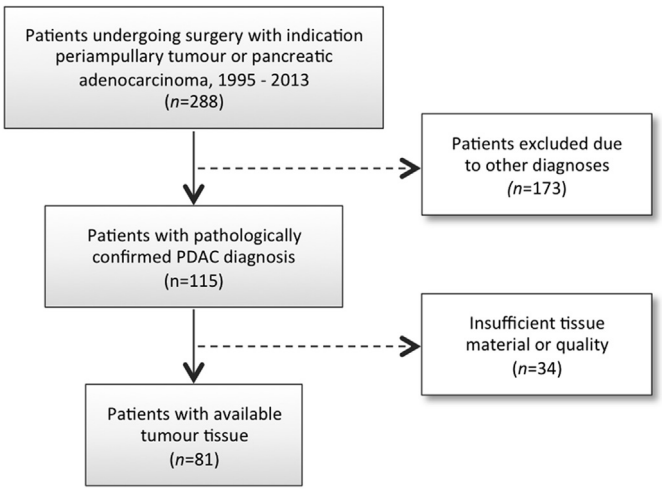

Fig. 1. Flow chart of patient selection. Illustration showing the stepwise selection process of patients for the study population. From the patients undergoing surgery for pancreatocoduodenectomy at Skåne University Hospital, only patients with pathology confirmed pancreatic ductal adenocarcinoma (PDAC) diagnosis were included. From 115 eligible patients, 81 patients had available tissue material and were included in the study. Among these, paired lymph node metastases were available for 39 patients.

Macrophage infiltration

Immunohistochemical staining of CD68 positive macrophages were performed using Dako Autostainer Plus (DakoCytomation, Glostrup, Denmark). Whole slide sections were deparaffinised and subjected to low pH antigen retrieval under pressure using EnVision FLEX (K8005, Dako) according to manufacturer's instructions. Section were subsequently incubated with anti-CD68 antibody (PG-M1, Dako; dilution 1:800), and visualised with DAB (EnVision FLEX, K8010, Dako). Following staining, whole slide digital images were captured using Aperio ScanScope Slide Scanner (Aperio Technologies, Leica Biosystems, Nussloch, Germany) and a 20× magnification lens. Fully automated image analyses with ImageScope software were done to include areas with cancer tissue. CD68 immunoreactivity was automatically sensored out and analysed through a positive pixel algorithm and fixed colour threshold values. Macrophage infiltration was quantified as positive pixels/μm^2 to correct for any differences in tissue area between sections.

Statistics

One patient was excluded due to tumour stage T4. Baseline characteristics were compared between patients with or without T2D by using χ^2 test for categorical variables and Mann-Whitney *U* test for continuous variables. For correlation between paired primary and metastatic leukocyte infiltration, Mann-Whitney *U* test was used for all patients as well as patients stratified according to T2D status. Leukocyte infiltration was grouped by percentiles in SPSS, and then divided into low/medium and high. In survival analyses, patients were followed from surgery until last follow-up or death, whichever came first, prior to January 1, 2015. Kaplan-Meier estimates were used to plot overall survival according to low-moderate and high tumour infiltration of macrophages, neutrophils or eosinophils, based on the tertile levels of infiltration among all patients or stratified according to type 2 diabetes status.

Please cite this article in press as: Karnevi E, et al., Intratumoural leukocyte infiltration is a prognostic indicator among pancreatic cancer patients with type 2 diabetes, Pancreatology (2017), https://doi.org/10.1016/j.pan.2017.11.003