Integrative Diabetes and Cardiovascular Diseases

β-cell Related Amyloidosis Localized to the Islets of Langerhans, Type II Diabetes Mellitus and Liponecrotic Pancreatopathitis in Rheumatoid Arthritis: A Postmortem Clinicopathologic Statistical Study of 234 Autopsy Patients

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Abstract

The aim of this study was to determine the prevalence of systemic AA amyloidosis (AAa), islet amyloidosis (IA) and liponecrotic pancreatitis (LnP) including acute liponecrotic (aLnP), acute relapsing liponecrotic (aRelLnP), and chronic liponecrotic pancreatitis (chrLnP) in rheumatoid arthritis (RA), and to analyse the possible relationship between them.

Patients and methods: At the National Institute of Rheumatology 11558 patients died between 1969 and 1998; among them 234 with RA, and all of them were autopsied. RA was confirmed clinically according to the criteria of the American College of Rheumatology (ACR). The diagnosis of DM was based on clinical data. Tissue samples of pancreas were available for histologic evaluation in 164 of 234 patients. AAa, IA and LnP were diagnosed histologically. Demographics of different patient cohorts were compared with the Student (Welch) t-probe. The relationships between AAa and IA, furthermore between IA and DM or LnP (including aLnP, aRelLnP, chrLnP) were analyzed by Pearson's chi-squared ($\chi^2$) test.

Results: AAa complicated RA in 42 (25.61%) of 164 patients. IA localized to the islets of Langerhans was observed in 16 (9.76%) of 164 pancreases. Clinically diagnosed DM was associated with RA in 31 (18.90%) of 164 patients. Pancreatitis with multiple liponecrotic foci (LnP) was found in 19 (11.58%) of 164 patients; aLnP existed in 9 (47.37%), aRelLnP in 4 (21.05%), and liponecrotic foci in combination with chronic fibrotic pancreatitis (chrLnP) in 6 (31.58%) of these 19 patients.

Discussion and conclusions: There was no significant difference between female and male RA patients associated with AAa, IA, DM and LnP. The age, sex and onset of disease did not influence basically the prevalence of AAa, IA, DM and LnP except male patients with IA, whose mean age at death was significantly higher than the general RA population. IA (fibrillar amyloid IAPP deposits -AIAPP) is related to the activity of β-cells and may presumably be a faulty product of β-cells (normal islets of Langerhans do not contain IA deposits). The progressive deposition of IAPP prohormon fragments inhibits the function of β-cells because of their toxic effect and/or blocking mechanically the blood supply of β-cells and they “die in their own product”. The significant correlation between IA and DM refers to a close connection between them, but not necessarily a direct cause and effect relationship; it may be an indirect result of damaged (apoptotic) β-cells. The early stage of IA is characterized by minimal IAPP deposits involving only a few islets, which represents a clinically latent DM, and the advanced stage of IA is characterized by massive IAPP...
Systemic AA amyloidosis (AAa) is one of the main chronic and progressive complications of rheumatoid arthritis (RA) - characterized by amyloid A deposition in various organs leading in most cases to renal and less frequently to cardiac insufficiency and death [1,2]. Systemic (secondary, reactive) amyloid A deposition (AA amyloidosis - AAa) occurs in a wide spectrum of chronic inflammatory diseases [3-5], such as

**Chronic microbial infections:** Tuberculosis [6,7], leprosy [8,9], fibrocystic lung diseases [10,11], bronchiectasis [7,12,13], lung abscess [6], chronic osteomyelitis [6,13], chronic xanthogranulomatous pyelonephritis [14-16], chronic mesenteric lymphadenitis [17], decubitus [7], etc.

**Chronic reactive inflammatory diseases:** Ankylosing spondylitis [7,13], psoriatic arthropathies [6,13,18], Reiter's syndrome, etc.

**Autoimmune (inflammatory) diseases:** Rheumatoid arthritis [7,13,19,20], juvenile chronic arthritis [6,7,13], adult Still’s disease, systemic lupus erythematoses [13], progressive systemic sclerosis [21], Crohn’s disease [22], ulcerative colitis [23], polymyositis [13], polymyalgia rheumatica or giant-cell arteritis (GCA) [24] etc. and in association with

**Chronic cachectic diseases or malignancies:** Renal cell carcinoma [13,25,26], ovarian carcinoma [27], hepatocellular adenoma [28-30], bronchial carcinoma [6,31], Hodgkin’s disease [32,33], cardiac (atrial) myxoma [34] etc.

Islet amyloidosis (IA) characterized by islet amyloid polypeptide (IAPP, also called amylin) prohormone fragment deposition localized to the islets of Langerhans is an isolated (localized) form of amyloidosis [35-40]. IA also represents a chronic progressive process [1,2,38,39], which may accompany RA as an associated disease independently from AAa, and may have clinical (diagnostic) significance in adult, non-insulin dependent, type II diabetes mellitus (DM) [1,2]. There is an agreement in the literature that the fibrillar amyloid IAPP (A-IAPP) deposition is toxic to insulin-producing β-cells, induces apoptosis and islet cell dysfunction resulting in type 2 DM [39,41].

The aim of this study was to determine the prevalence of AAa, IA and liponecrotic pancreatitis (LnP) including acute liponecrotic (aLnP), acute relapsing liponecrotic (aRelLnP), and chronic liponecrotic pancreatitis (chrLnP) in RA, and to analyse the possible relationship between them.

**Patients and Methods**

At the National Institute of Rheumatology 11558 patients died between 1969 and 1998; among them 234 with RA, and all of them were autopsied. RA was confirmed clinically according to the criteria of the American College of Rheumatology (ACR) [42]. The diagnosis of DM was based on clinical data.

AAa and IA (amyloid A and IAPP deposition) was diagnosed histologically according to Romhányi [43] by a modified (more sensitive) Congo red staining [44]. AAa and IA deposits were identified in serial sections by immunohistochemical and histochemical methods [45,46].

Tissue samples of pancreas were available for
histologic evaluation in 164 of 234 patients. Prevalence and histological patterns of pancreatitis were determined at autopsy and characterized histologically [47,48]. Demographics of different patient cohorts were compared with the Student (Welch) \(t\)-probe [49]. The relationships between AAa and IA, furthermore between IA and DM or LnP (including aLnP, aRelLnP, chrLnP) were analyzed by Pearson's chi-squared (\(\chi^2\)) test [49].

Glossary of definitions

Prevalence of AAa was specified histologically based on the presence of amyloid A in blood vessels of different calibers or in different tissue structures of five organs (heart, lung, liver, kidney and pancreas) in each patient [50]. Prevalence of IA concerns the presence of islet amyloid polypeptide (IAPP) prohormone fragment deposition localized to the islets of Langerhans [2].

**Histologic patterns of pancreatitis:** Liponecrotic pancreatitis (LnP) – multiple acinar liponecrotic foci, with or without inflammatory reaction, with or without hemorrhage.

**Acute liponecrotic pancreatitis (aLnP):** Acinar liponecrotic foci usually in the same stage and similar size of necrosis, with or without inflammatory reaction, with or without hemorrhage.

**Acute relapsing liponecrotic pancreatitis (aRelLnP):** Acinar liponecrotic foci in different stage and size of necrosis, with or without inflammatory reaction, hemorrhage, calcification (saponification) or liquefaction (pseudocyst formation).

**Chronic pancreatitis (chrP):** Multifocal or diffuse fibrotic interstitial pancreatitis with more or less pronounced glandular atrophy, with or without ductal changes: pluges of inspissated secretion of exocrine glands, ductal dilatation, ductal proliferation and/or epithelial metaplasia.

**Chronic liponecrotic pancreatitis (chrLnP):** liponecrotic foci with histological characteristics of chrP.

Edematous inflammatory pancreatitis or “serous” infection associated pancreatitis (eIP), usually a mild diffuse edematous inflammatory interstitial pancreatitis without acinar cell necrosis or hemorrhage.

The prevalence of chrP and eIP were not evaluated in this study.

Results

AAa complicated RA in 42 (25.61%) of 164 patients. \(\beta\)-cell related IA localized to the islets of Langerhans was observed in 16 (9.76%) of 164 pancreases. Clinically diagnosed DM was associated with RA in 31 (18.90%) of 164 patients. Pancreatitis with multiple liponecrotic foci (LnP) was found in 19 (11.58%) of 164 patients; aLnP existed in 9 (47.37%), aRelLnP in 4 (21.05%), and liponecrotic foci in combination with chronic fibrotic pancreatitis (chrLnP) in 6 (31.58%) of these 19 patients.

Demographics, onset and duration of RA complicated by AAa or associated with IA, DM, LnP (including aLnP, aRelLnP or chrLnP) are summarized in table 1.

Comparing the age, sex, onset of RA, and duration of disease there was no significant difference between female and male RA patients (n = 164) with AAa (n = 42), IA (n = 16), DM (n = 31) and LnP (n = 19, except male patients with IA, whose mean age at death was significantly higher (73.57 years versus 65.57; \(p < 0.035\)) in comparison with average age of the others.

The mean age of RA patients associated with aLnP was significantly higher at the onset of disease (65.06 years versus 51.43; \(p < 0.00025\)), and these patients died significantly earlier (4.31 years versus 14.64; \(p < 0.026\)) than the average of others. The tendency was similar both in women (64.08 years versus 50.82; \(p < 0.00002\), and 2.92 years versus 15.32; \(p < 0.089 - \text{NS}\)) and men (68.00 years versus 52.85; \(p < 0.556 - \text{NS}\), and 8.50 years versus 13.05; \(p < 0.089 - \text{NS}\)).

RA started earlier in patients with aRelLnP (48.00 years versus 51.43; \(p < 0.046\), with longer disease duration (19.75 years versus 14.64; \(p < 0.563 - \text{NS}\)) than in the average of others. The tendency regarding the onset of RA and disease duration was similar both in women (46.00 years versus 50.82; \(p < 0.00002\), and 19.00 versus 15.32 – \text{NS}) and in men (50.00 years versus 52.85; \(p < 0.852 - \text{NS}\)).

The statistical links (“p” values of significance) between RA patients complicated by AAa or associated with IA, DM, LnP (including aLnP, aRelLnP or chrLnP) are summarized in table 2.

AAa was associated with IA in 2 (4.76%) with DM in 6 (14.28%) with LnP in 6 (14.28%) with aLnPA in none (0%) with aRelLP in 2 (4.76%), and with chrLP in 4 (9.52%) of 42 cases. The relationship between AAa and IA was
not significant, although the association coefficient was negative (negative value of association coefficient: -0.4433, $\chi^2 = 0.9278$, $p < 0.335$). AAa did not influence the prevalence of DM, LnP, aLnP, aRelLnP or chrLnP (Table 3).

IA was associated with clinically diagnosed DM in 9 (56.25%) of 16 patients. There was a positive and significant correlation between clinically diagnosed IA and DM (association coefficient: 0.7608, $\chi^2 = 16.1324$, $p < 0.00006$). IA was present without the clinical diagnosis of DM in 7 (43.75%) of 16 patients. The relationship between IA and clinically not diagnosed DM was also positive and significant (association coefficient: 0.6014, $\chi^2 = 7.1407$, $p < 0.007$).

IA was associated with LnP in 3 (18.75%) of 16 patients and without exception only with aLnP or aRelLnP (IA was not associated with chrLnP). The relationship between IA and aLnP (association coefficient: 0.3762, $\chi^2 = 0.2016$, $p < 0.653$) or IA and aRelLnP (association coefficient: 0.5263, $\chi^2 = 0.0350$, $p < 0.851$) was not significant. The link between IA and chrLnP was inverse, with negative the association coefficient and without significant relationship (negative value of association coefficient: -1.0000, $\chi^2 = 0.0014$, $p < 0.970$).

DM associated with LnP in 6 (56.25%) of 31 patients, and in all of these cases exclusively with aLnP or aRelLnP (DM was not associated with chrLnP). The relationship between DM and aLnP (association coefficient: 0.5826, $\chi^2 = 4.0526$, $p < 0.044$) was significant. The link between DM and aRelLnP (association coefficient: 0.3796, $\chi^2 = 0.1510$, $p < 0.697$) or between DM and chrLnP (negative value of association coefficient: -1.0000, $\chi^2 = 0.4538$, $p < 0.50$), was not significant, even in this latter inverse with negative association coefficient.

The statistical links ("p" values of significance) between coexistant AAa, IA, DM and LnP (including aLnP, aRelLnP or chrLnP) are summarized in table 3.

The distribution of $\alpha$- and $\beta$- cells in the islets of Langerhans is demonstrated in figures 1 and 2 and the progressive process of IA in figures 3-15. Figures 3-6 show an early stage of deposition, Figure 7 an advanced, Figures 8-10 a late, and Figures 11-15 the terminal stage of amyloid fibril deposition of IAPP.

![Figure 1](image1.png)  
*Figure 1*: Brown colour of immunohistochemical reaction indicate the normal distribution of $\alpha$-cells in islets of Langerhans. $\alpha$-cells are positive for anti-human Glucagon (a): hu Glucagon dilution 1:150 polyclonal antibody A0565; DAKO, Glostrup, Denmark, was used to detect the $\alpha$-cells, Streptavidin-biotin-complex/horseradish peroxidase reaction, x100; (b) Same as (a) x200.

![Figure 2](image2.png)  
*Figure 2*: Brown colour of immunohistochemical reaction indicate the normal distribution of $\beta$-cells in islets of Langerhans. $\beta$-cells are positive for anti-human Insulin. (a) a-hu Insulin diluted [polyclonal antibody A0564; DAKO, Glostrup, Denmark], Streptavidin-biotin-complex/horseradish peroxidase reaction, x100; (b) Same as (a) x200.
Original magnifications correspond to the 24×36 mm transparency slide; the correct height: width ratio is 2:3. The printed size may be different, therefore it is necessary to indicate the original magnifications.

Minimal deposits of AIAPP localized to the islets of Langerhans in figures 3-6.

Normal islet of Langerhans without AIAPP and an adjacent one with moderate deposits of AIAPP are demonstrated in figure 7a-7f.

**Figure 3:** Pancreas, with minimal deposits of AIAPP localized to islets of Langerhans, early stage of IA. 
(a) H-E, x100; (b) Same as Figure (a) x200.

**Figure 4:** Pancreas, with minimal deposits of AIAPP localized to islets of Langerhans, early stage of IA. 
(a) PAS, x100; (b) Same as Figure (a) x200.

**Figure 5:** Pancreas, with minimal deposits of AIAPP localized to islets of Langerhans, early stage of IA. 
(a) Congo red staining, without alcoholic differentiation, covered with gum arabic, x100; 
(b) Same as Figure (a) x200.
Figure 6: Pancreas, with minimal deposits of AIAPP localized to islets of Langerhans, early stage of IA. The apple green birefringence corresponds to AIAPP deposits, “white” birefringence is caused by paraffin remnants due to imperfect deparaffinization. The minimal AIAPP deposits in the islets of Langerhans may be easily overlooked under low magnification because of weak birefringence, or using a less sensitive Congo red staining than Romhányi’s method. (a) Congo red staining, without alcoholic differentiation, covered with gum arabic, viewed under polarized light, x100; (b) same as Figure (a) x200.

Figure 7: Islets of Langerhans without AIAPP deposits and with moderate amount of AIAPP deposits, advanced stage of IA. (a) H-E, x100; (b) Same as (a) PAS, x100.

Figure 7c: Congo red staining according to Romhányi, without alcoholic differentiation, covered with gum arabic, viewed under polarized light, x100.

Figure 7d: α-cells are positive for anti-human Glucagon a-hu Glucagon dilution 1:150; [polyclonal antibody A0565; DAKO, Glostrup, Denmark], to detect the α-cells, Streptavidin-biotin-complex/horseradish peroxidase reaction, x100.
**Figure 7e:** β-cells are positive for anti-human insulin.

α-hu Insulin diluted [polyclonal antibody A0564; DAKO, Glostrup, Denmark], Streptavidin-biotin-complex/horseradish peroxidase reaction, x100.

**Figure 7f:** Islet amyloid polypeptide – AIAPP deposits are negative for P-component (note the positive cross reaction of elastic fibres).

α-hu amyloid P-component dilution, 1:300; [polyclonal antibody A0302; DAKO, Glostrup, Denmark], Streptavidin-biotin-complex/horseradish peroxidase reaction

Massive deposits of AIAPP localized to the islets of Langerhans are demonstrated in figures 8–10.

The number of α-, β-, γ-, and δ-cells decreased in correlation with the amount of deposited hormone-related amyloid. In advanced stages of islet amyloidosis only the β-cells are microscopically detectable; the AIAPP deposits are connected to the β-cells.

**Figure 8 a,b:** Pancreas, with massive deposits of AIAPP located to the islets of Langerhans, late stage of IA.

(a) HE, x100; (b) Same as figure (a) x200.

**Figure 9:** (a) PAS, x 100 (b) Same as (a) x200.
Diffuse deposition of AIAPP localized to the islets of Langerhans in terminal stage of progressive deposition is demonstrated in figures 11–15. In these RA patients AIAPP deposition is associated with AAa (note adjacent pancreatic arteriole).

**Figure 10:** (a) Congo red staining, without alcoholic differentiation, covered with gum arabic, under polarized light, x 100 (b) Same as (a) x200.

**Figure 11:** Pancreas, massive diffuse deposits of AIAPP localized to islets of Langerhans, terminal stage of IA. (a) H-E, x100; (b) Same as Figure (a) x200.

**Figure 12:** Pancreas, massive diffuse deposits of AIAPP localized to islets of Langerhans, terminal stage of IA. (a) PAS, x100; (b) Same as Figure (a) x200.
**Figure 13:** Pancreas, massive diffuse deposits of AIAPP localized to islets of Langerhans, terminal stage of IA. 
(a) Congo red staining, without alcoholic differentiation, covered with gum arabic, x100; (b) Same as Figure (a) x200.

**Figure 14:** Pancreas, massive deposits of AIAPP localized to islets of Langerhans, terminal stage of IA. 
(a) Congo red staining, without alcoholic differentiation, covered with gum arabic and viewed under polarized light, x100; (b) Same as (a) x200.

**Figure 15:** Pancreas, amyloid A deposits in the wall of arterioles (positive staining for a-hu amyloid A-component) and massive, diffuse AIAPP deposits (negative staining for a-hu amyloid A-component) localized to the islets of Langerhans, terminal stage of IA. The AIAPP deposits are resistant to performate pretreatment (for 1 - 10 sec), resistant/sensitive (for 15 - 20 sec) and resistant to KMnO4 oxidation (for 30 sec – 10 min) [45,46].

(a) a-hu amyloid A-component 1:100 [monoclonal antibody MO759; DAKO, Glostrup, Denmark], Streptavidin-biotin-complex/horseradish peroxidase reaction, x100; (b) Same as (a) x200.

**Discussion**

IA (fibrillar amyloid IAPP deposits) is regarded as a key factor in the pathogenesis of type 2 diabetes [38,39]. IAPP is a normal β-cell component [51,52], synthetized by the β-cells, co secreted and released with insulin [52,53], unique (other than insulin) [35], and in type 2 diabetes is deposited as amyloid fibrils in the islets of Langerhans [51].

The amyloid fibrils of IAPP (AIAPP) are cytotoxic to β-cells [41,54], and progressively destroy the insulin-producing beta cells (apoptosis) [39,55,56]. IAPP may cause insulin resistance opposing the action of insulin in
Table 1: Sex, mean age with SD, range, onset and disease duration of RA patients with AAa (n=42), IA (n=16), DM (n=31) and LnP (n=19) (including aLnP n=9, aRelLnP n=4 or chrLnP n=6).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of autopsies</th>
<th>Mean age in years at death ± SD (in years)</th>
<th>Range</th>
<th>Mean age at onset of disease ± SD</th>
<th>Disease duration (in years) mean ± SD</th>
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<tbody>
<tr>
<td>RA patients</td>
<td>164</td>
<td>65.63 ± 12.45</td>
<td>16–88</td>
<td>51.43 ± 16.61</td>
<td>14.64 ± 10.88</td>
</tr>
<tr>
<td>Female</td>
<td>113</td>
<td>65.65 ± 11.98</td>
<td>16–88</td>
<td>50.82 ± 15.65</td>
<td>15.32 ± 11.22</td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td>65.57 ± 13.44</td>
<td>19–88</td>
<td>52.85 ± 18.62</td>
<td>13.05 ± 9.83</td>
</tr>
<tr>
<td>With AAa</td>
<td>42 of 164</td>
<td>63.40 ± 14.49</td>
<td>19–88</td>
<td>46.28 ± 17.00</td>
<td>17.28 ± 10.00</td>
</tr>
<tr>
<td>Female</td>
<td>32</td>
<td>64.94 ± 9.34</td>
<td>44–88</td>
<td>46.87 ± 15.12</td>
<td>17.97 ± 10.81</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>58.50 ± 22.81</td>
<td>19–88</td>
<td>44.00 ± 22.72</td>
<td>14.63 ± 5.02</td>
</tr>
<tr>
<td>With IA</td>
<td>16 of 164</td>
<td>70.19 ± 10.15</td>
<td>45–83</td>
<td>55.69 ± 13.05</td>
<td>14.50 ± 8.44</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>67.44 ± 11.42</td>
<td>45–82</td>
<td>55.00 ± 14.23</td>
<td>14.00 ± 8.99</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>73.57 ± 6.84</td>
<td>67–83</td>
<td>56.83 ± 10.70</td>
<td>15.33 ± 7.36</td>
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<tr>
<td>With DM</td>
<td>31 of 164</td>
<td>69.10 ± 9.13</td>
<td>47–83</td>
<td>55.84 ± 11.70</td>
<td>14.52 ± 9.42</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>67.50 ± 8.35</td>
<td>47–82</td>
<td>64.76 ± 11.61</td>
<td>14.19 ± 10.46</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>72.00 ± 9.75</td>
<td>48–83</td>
<td>58.13 ± 11.54</td>
<td>15.00 ± 6.69</td>
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<tr>
<td>With LnP</td>
<td>19 of 164</td>
<td>67.58 ± 13.61</td>
<td>32–87</td>
<td>58.41 ± 13.08</td>
<td>10.59 ± 9.22</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>68.38 ± 11.76</td>
<td>51–87</td>
<td>57.68 ± 13.44</td>
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<tr>
<td>Male</td>
<td>6</td>
<td>65.83 ± 16.81</td>
<td>32–82</td>
<td>60.00 ± 12.08</td>
<td>12.60 ± 7.45</td>
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<tr>
<td>With aLnP</td>
<td>9 of 19</td>
<td>65.22 ± 15.88</td>
<td>32–83</td>
<td>65.06 ± 12.76</td>
<td>4.31 ± 4.63</td>
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<tr>
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<td>7</td>
<td>67.00 ± 12.17</td>
<td>51–83</td>
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<td>2</td>
<td>61.67 ± 21.01</td>
<td>32–78</td>
<td>68.00 ± 7.00</td>
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<tr>
<td>With aRelLnP</td>
<td>4 of 19</td>
<td>67.75 ± 9.91</td>
<td>58–82</td>
<td>48.00 ± 8.97</td>
<td>19.75 ± 2.95</td>
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<tr>
<td>Female</td>
<td>2</td>
<td>65.00 ± 7.00</td>
<td>58–72</td>
<td>46.00 ± 3.00</td>
<td>19.00 ± 4.00</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>70.50 ± 11.50</td>
<td>59–82</td>
<td>50.00 ± 12.00</td>
<td>20.50 ± 0.50</td>
</tr>
<tr>
<td>With chrLnP</td>
<td>6 of 19</td>
<td>64.17 ± 17.81</td>
<td>32–87</td>
<td>48.00 ± 10.65</td>
<td>18.50 ± 12.54</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>71.00 ± 12.83</td>
<td>54–87</td>
<td>42.67 ± 6.13</td>
<td>23.00 ± 11.34</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>50.50 ± 18.50</td>
<td>32–69</td>
<td>64.00 ± 0.00</td>
<td>5.00 ± 0.00</td>
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RA: Rheumatoid Arthritis  
AAa: systemic AA amyloidosis  
IA: pancreatic IA amyloidosis  
LnP: Liponecrotic Pancreatitis  
aLnP: acute Liponecrotic Pancreatitis  
aRelLnP: acute Relapsing Liponecrotic Pancreatitis  
chrLnP: chronic Liponecrotic Pancreatitis  
SD: Standard deviation

Peripheral tissues [53], but others deny any physiologically important extra-islet metabolic effects of it [38].

Our data indicate that IAPP-related amyloidosis localized to the islets of Langerhans is a progressive and cumulative process, like any form and type of systemic or localized amyloidosis [1]. The ratio of islets of Langerhans with amyloid deposits and the amount of deposited prohormone fragments (IAPP) per islet depends on the stage of insular amyloidosis. According to our data IAPP deposition is a localized process connected to the β-cells in agreement with others [35,53-56].

Recent studies confirm the reduced number and abnormal function of β-cells [57-59] accompanied with increased insulin secretion by the remaining β-cells [58]. In our patients the number of α-, β-, γ-, and δ-cells decreased in proportion to the amount of deposited IAPP, and the number of apoptotic cells increased. IAPP deposits inhibit the glucagon secretion of α-cells [60]. For...
Table 2: Statistical correlations (“p” values of significance) between female and male RA patients with AAa or associated with IA, DM, LnP (including aLnP, aRelLnP or chrLnP).

<table>
<thead>
<tr>
<th>RA patients n=234 – available pancreas n=164</th>
<th>Age</th>
<th>Onset of disease</th>
<th>Disease duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA pts. n=164 versus pts. with AAa n=42 of 164</td>
<td>p&lt; 0.371</td>
<td>p&lt; 0.163</td>
<td>p&lt; 0.102</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=32 of 42</td>
<td>p&lt; 0.742</td>
<td>p&lt; 0.251</td>
<td>p&lt; 0.221</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=10 of 42</td>
<td>p&lt; 0.388</td>
<td>p&lt; 0.529</td>
<td>p&lt; 0.356</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with IA n=16 of 164</td>
<td>p&lt; 0.119</td>
<td>p&lt; 0.952</td>
<td>p&lt; 0.257</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=9 of 16</td>
<td>p&lt; 0.678</td>
<td>p&lt; 0.688</td>
<td>p&lt; 0.422</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=7 of 16</td>
<td>p&lt; 0.035</td>
<td>p&lt; 0.549</td>
<td>p&lt; 0.496</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with DM n=31 of 164</td>
<td>p&lt; 0.078</td>
<td>p&lt; 0.954</td>
<td>p&lt; 0.119</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=20 of 31</td>
<td>p&lt; 0.410</td>
<td>p&lt; 0.723</td>
<td>p&lt; 0.245</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=11 of 31</td>
<td>p&lt; 0.092</td>
<td>p&lt; 0.522</td>
<td>p&lt; 0.333</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with LnP n=19 of 164</td>
<td>p&lt; 0.567</td>
<td>p&lt; 0.129</td>
<td>p&lt; 0.071</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=13 of 18</td>
<td>p&lt; 0.456</td>
<td>p&lt; 0.112</td>
<td>p&lt; 0.155</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=6 of 18</td>
<td>p&lt; 0.974</td>
<td>p&lt; 0.915</td>
<td>p&lt; 0.328</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with aLnP n=9 of 19</td>
<td>p&lt; 0.500</td>
<td>p&lt; 0.00025</td>
<td>p&lt; 0.026</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=7 of 9</td>
<td>p&lt; 0.921</td>
<td>p&lt; 0.00002</td>
<td>p&lt; 0.089</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=2 of 9</td>
<td>p&lt; 0.003</td>
<td>p&lt; 0.556</td>
<td>p&lt; 0.238</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with aRelLnP n=4 of 19</td>
<td>p&lt; 0.738</td>
<td>p&lt; 0.046</td>
<td>p&lt; 0.563</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=2 of 4</td>
<td>p&lt; 0.942</td>
<td>p&lt; 0.521</td>
<td>p&lt; 0.316</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=2 of 4</td>
<td>p&lt; 0.742</td>
<td>p&lt; 0.00006</td>
<td>p&lt; 0.852</td>
</tr>
<tr>
<td>RA pts. n=164 versus pts. with chrLnP n=6 of 19</td>
<td>p&lt; 0.862</td>
<td>p&lt; 0.633</td>
<td>p&lt; 0.621</td>
</tr>
<tr>
<td>Female n=113 of 164 versus n=4 of 6</td>
<td>p&lt; 0.524</td>
<td>p&lt; 0.440</td>
<td>p&lt; 0.190</td>
</tr>
<tr>
<td>Male n=51 of 164 versus n=2 of 6</td>
<td>p&lt; 0.564</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

RA: Rheumatoid Arthritis
AAa: systemic AA amyloidosis
IA: pancreatic IA amyloidosis
LnP: Liponecrotic Pancreatitis
aLnP: acute Liponecrotic Pancreatitis
aRelLnP: acute Relapsing Liponecrotic Pancreatitis
chrLnP: chronic Liponecrotic Pancreatitis

normal function the harmonic interactions of α- and β-cells are essential [61]. Unfortunately, the kinetics of β-cell mass reduction and dysfunction as well as the underlying mechanisms remain unclear [62].

The high value of association coefficient (c = 0.7608) and the positive and significant correlation between IA and DM ($\chi^2 = 16.1324, p < 0.00006$) refer to a very close connection, but this does not necessarily mean a cause and effect relationship; an associated phenomenon seems more likely. Several etiologic factors (insulin resistance, defective receptors, hyperglycemia, hyperlipidemia, antigens etc.) may play a role in the development of DM (see below).

The etiology of DM is probably polygenetic, influenced by environmental effects: [63,64]. For this reason between identical twins the concordance is only 90-95% [65]. It is generally accepted that DM is caused by insulin resistance of peripheral receptors in skeletal muscle, liver, adipose tissue. Insulin resistance is also present in the cells of islets of Langerhans. The alpha-cells produce more glucagon, because of insulin resistance and lack of glucose within the cells [66]. According to some theories the defect of insulin receptors is determined genetically [67]. Some other popular theories include sparing genes [68,69], hyperglycemia, hyperlipidemia [70], islet amyloidosis [35,36,55] or low birth weight [71].

In our opinion IAPP deposition is not the cause of DM, rather an inherent (accompanying) phenomenon or faulty product of the overloaded and exhausted β-cells.
### Table 3: The statistical links ("p" values of significance) of coexistent complications and associated diseases in 164 RA patients.

<table>
<thead>
<tr>
<th>Prevalence of coexistent complications &amp; assoc. disease</th>
<th>IA n=16</th>
<th>DM n=31</th>
<th>LnP n=19</th>
<th>aLnP n=9</th>
<th>aRelLnP n=4</th>
<th>chrLnP n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAa n=42 of 164</td>
<td>c =-0.4433*</td>
<td>c =-0.2145*</td>
<td>c =-1.000*</td>
<td>c =0.5000</td>
<td>c=0.7266</td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=-0.9278*</td>
<td>χ²=-0.7850*</td>
<td>χ²=2.010*</td>
<td>χ²=0.3042</td>
<td>χ²=3.5005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.335</td>
<td>p&lt; 0.375</td>
<td>p&lt; 0.156</td>
<td>p&lt; 0.581</td>
<td>p&lt; 0.061</td>
<td></td>
</tr>
<tr>
<td>IA n=16 of 164</td>
<td>c=0.7608</td>
<td>c=0.3112</td>
<td>c=0.3762</td>
<td>c=0.5263</td>
<td>c =-1.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=16.1324</td>
<td>χ²=0.2824</td>
<td>χ²=0.2016</td>
<td>χ²=0.0350</td>
<td>χ²=-0.0014*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.0006</td>
<td>p&lt; 0.653</td>
<td>p&lt; 0.851</td>
<td>p&lt; 0.970</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM n=31 of 164</td>
<td>c=0.3779</td>
<td>c=0.5826</td>
<td>c=0.3796</td>
<td>c=-1.0000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=2.2527</td>
<td>χ²=4.0526</td>
<td>χ²=0.1510</td>
<td>χ²=-0.4538*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.133</td>
<td>p&lt; 0.044</td>
<td>p&lt; 0.697</td>
<td>p&lt; 0.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LnP n=19 of 164</td>
<td>c=1.0000</td>
<td>c=1.0000</td>
<td>c=1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=63.8264</td>
<td>χ²=23.0675</td>
<td>χ²=38.9911</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.00000</td>
<td>p&lt; 0.00000</td>
<td>p&lt; 0.00000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aLnP n=9 of 19</td>
<td>c =-1.000*</td>
<td>c =-1.000*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=-0.3616*</td>
<td>χ²=-0.0097*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.5476</td>
<td>p&lt; 0.755</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aRelLnP n=4 of 19</td>
<td>c =-1.000*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>χ²=-0.9093*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt; 0.340</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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aLnP: acute Liponecrotic Pancreatitis  
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chrLnP: chronic Liponecrotic Pancreatitis  
* - Asterisk designates a negative value of association coefficient.

(in healthy individuals there is no AIAPP in the islets of Langerhans). According to our interpretation the early stage of IA (involving only a few islets with minimal IAPP deposits) represents a clinically latent (potential) DM and the advanced stage of it (involving most of the islets with massive IAPP deposits) corresponds to clinically manifest DM.

Apart from this the positive and significant correlation between IA and clinically not diagnosed DM, IA may be a good indicator of potential DM in the latent stage of disease. This correlation may help recognize DM in its early stage. For this reason we recommend that all biopsy material and surgical specimens of pancreas to be tested for IA or IAPP deposition. Our data do not support the theory that IAPP influences the prevalence of LnP, including aLnP, aRelLnP or chrLnP, in contrast with the opinion of Noel et al. [70] or Lai et al. [71], only patients with DM had a higher risk of acute pancreatitis, more precisely of aLnP, based on the strong relationship between them (association coefficient = 0.58269, $\chi^2 = 4.0526 \ p < 0.044$) [72,73].

Systemic or localized types of amyloidosis may exist simultaneously side by side or may be present independently from each other. AAa and IA are independent phenomena which may coexist in RA, based on the negative association coefficient and absence of significant relationship. Severe AAa may involve blood vessels of different calibers of the pancreas and should be regarded as important factors in the pathogenesis of aRelLnP [74]. The role of severe AAa in pathogenesis of fatal acute pancreatitis is confirmed by others as well [75-77].

Authors suggest the role of “insulitis” in DM...
characterized by increased number of intra-islet macrophages, cytokines (including increased interleukin (IL)-1 beta expression), β-cell apoptosis, immune cell infiltration, IA deposits and fibrosis [78-81]. In our patient population “insulitis” was present only in cases associated with generalized lethal septic infection and edematous inflammatory pancreatitis (eIP, “serous” infection associated pancreatitis). The presence of macrophages in AIAAPP deposits (with cytokines etc.) is a normal tissue reaction to pernicious IAPP deposits, rather than cause of DM.

There was no significant difference between female and male RA patients associated with AAa, IA, DM and LnP. The age, sex and onset of disease did not influence basically the prevalence of AAa, IA, DM and LnP except male patients with IA, whose mean age at death was significantly higher (73.57 years versus 65.57; \( p < 0.035 \)) than the general RA population.

**Conclusions**

IA (fibrillar amyloid IAPP deposits) is related to the activity of β-cells and may presumably be a faulty product of β-cells (normal islets of Langerhans do not contain IA deposits). The progressive deposition of IAPP prohormon fragments inhibits the function of β-cells because of their toxic effect and/or blocking mechanically the blood supply of β-cells and they “die in their own product”. The significant correlation between IA and DM refers to a close connection between them, but not necessarily a direct cause and effect relationship; it may be an indirect result of demaged (apoptotic) β-cells. The early stage of IA is characterized by minimal IAPP deposits involving only a few islets, which represents a clinically latent DM, and the advanced stage of it is characterized by massive IAPP deposits involving most of the islets, which correspond to clinically manifest DM.

Based on the positive and significant correlation between IA and clinically not diagnosed DM, IA may be a good indicator of potential DM in the latent stage of disease. Therefore we recommend that all biopsy material and surgical specimens of pancreas to be tested for IA or IAPP deposition.

**References**


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