Incidence of Adipose Tissue Alterations in First-Line Antiretroviral Therapy

The LipoICoNa Study

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Background: Adipose tissue alterations (ATAs) are a frequent untoward effect of antiretroviral therapy, the causes of which remain incompletely explained.

Objectives: To assess the incidence of ATAs and to identify the associated risk factors in patients infected with human immunodeficiency virus type 1 starting their first-line antiretroviral treatment.

Methods: In a multicenter investigation designed to study issues related to the treatment of patients starting antiretroviral therapy, physicians were requested to assess the presence of ATAs at enrollment and every 6 months thereafter. The ATAs were considered altogether and grouped as fat loss (lipoatrophy), adipose tissue accumulation (lipo-hypertrophy), and combined forms.

Results: A total of 655 patients were followed up for a median of 86 weeks; 128 patients (19.6%) were diagnosed as having at least 1 morphologic alteration during the study. Female gender and positivity for hepatitis C virus were independently linked to an increased risk of developing morphologic alterations. Age was another independent correlate of risk of developing ATAs. To have been infected through drug injection was a correlate of reduced risk of ATAs. Stavudine exposure was predictive at borderline statistical significance of lipoatrophy (but not of the other forms), and indinavir exposure was associated with a significantly higher risk of developing combined forms. Patients who started therapy with 2 nucleoside reverse transcriptase inhibitors and subsequently added a protease inhibitor during the follow-up had a significantly higher risk of having ATAs compared with patients who continued taking 2 nucleoside reverse transcriptase inhibitors up to the end of follow-up.

Conclusions: Different types of ATAs might derive from distinct pathways and multifactorial causes. Adipose tissue alterations are a frequent and relatively early finding during first-line antiretroviral therapy.

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Adipose tissue alterations (ATAs) are a complex spectrum of manifestations that have been alternatively attributed to direct drug toxicity and to immunologic or endocrinologic events triggered by the treatment; nevertheless, their cause and determinants have not yet been defined.

Patients receiving protease inhibitors (PIs) have a high prevalence of morphologic and metabolic alterations with an increasing trend that parallels duration of therapy. However, PI-naive patients receiving treatment with nucleoside reverse transcriptase inhibitors (NRTI) may also present the entire spectrum of alterations.

In this context, the role of each drug or drug class in causing adipose tissue disorders is still debated. In particular, stavudine appeared in several studies as exerting an independent role in increasing the risk of ATAs. These findings could not be confirmed in other studies. Considering only investigations on first-line treatments, an independent role of stavudine in causing ATAs was reported by Mauss and colleagues but not confirmed in 3 other cohorts. However, in one of these studies, a longer exposure to stavudine was associated with an increased risk of developing lipoatrophy. The heterogeneity of the populations involved, of the study design, and of the statistical analysis performed may represent significant obstacles for comparison of the data coming from different investigations. For example, female patients are underrepresented or not represented at all in some studies; moreover, the role of genetic and diet factors in predisposing to metabolic and adipose tissue abnormalities and their
differences in populations living in different countries are still poorly assessed. Furthermore, social and behavioral factors that could influence adherence to therapy and comorbidity factors, such as hepatitis C virus (HCV) infection, are frequently not considered.

The present study was designed to assess the incidence rate of ATAs in patients starting antiretroviral therapy (ART) for the first time, to identify the risk factors linked to ATA development, and to evaluate the role of each drug used. Moreover, taking into account observations suggesting that lipo hypertrophy and lipoatrophy may have different correlates of risk, we tried to identify the factors associated with the development of morphologic alterations separately for each type of ATA.

### METHODS

The Italian Cohort of Antiretroviral-Naive Patients (IcoNa) is a multicentric observational study. As reported elsewhere, the database includes clinical and demographic characteristics, data on laboratory markers, and dates of starting and stopping each drug. Moreover, taking into account observations suggesting that lipo hypertrophy and lipoatrophy may have different correlates of risk, we tried to identify the factors associated with the development of morphologic alterations separately for each type of ATA.

### RESULTS

The study included 655 patients followed up after the beginning of ART for a median time of 86 weeks (interquartile range, 52-107 weeks).

Table 1 reports the demographic data and the baseline laboratory values. Most patients (n=426 [65%])
started a combination of 3 or more drugs that included a PI. None of the patients enrolled in the study received non-NRTIs in their first-line treatment or for more than 6 months during the follow-up.

Figure 1 shows the proportion of patients initiating each drug as a component of their first-line treatment and the percentage of patients who took each drug during the follow-up. Zidovudine was the drug most frequently initiated (70.2%), but by the end of follow-up, lamivudine was the most frequently used (85.2%). Indinavir was the PI most frequently used both at the date of ART initiation (32.2%) and by the end of follow-up (45.5%).

Figure 2 shows the duration of exposure to each individual drug. Again, lamivudine (median, 23.4 months; interquartile range, 12.9-32.8 months), zidovudine (median, 15.7 months; interquartile range, 9.2-23.7 months), and indinavir (median, 12.8 months; interquartile range, 7.0-21.3 months) were, on average, the drugs with the longest duration of exposure. The proportions of patients initiating each NRTI were similar in subjects starting dual nucleoside therapy and in subjects treated with combinations that included a PI. At the end of the follow-up, the proportion of patients who had used each drug and the duration of exposure were similar in patients starting HAART and patients who started a suboptimal dual therapy (data not shown).

Of the 655 patients, 128 (19.5%) were diagnosed as having at least 1 morphologic alteration during the study follow-up. Figure 3 reports the number of the alterations observed in each body region, expressed as a percentage of the total number of patients studied. Abdominal fat accumulation was the most frequently observed event (72 patients). Adipomasty was observed in 15
women. The body sites most frequently showing loss of adipose tissue were lower limbs (48 patients) and cheeks (28 patients). The overall incidence of morphologic alterations was 12.7 per 100 person-years (95% confidence interval [CI], 10.6-15.1). Fat accumulation alone, with 60 cases and an incidence of 5.5 per 100 patient-years (95% CI, 4.2-7.1), was the most frequently observed alteration, followed by fat loss (43 cases, 3.8 per 100 patient-years; 95% CI, 2.8-5.2) and a combination of fat loss and fat accumulation (29 cases, 2.6 per 100 patient-years; 95% CI, 1.7-3.7).

The time to the diagnosis of the various alterations was variable. Abdominal fat accumulation tended to occur earlier than any other alteration (14% of patients by week 112; 95% CI, 11%-17%), followed by adipomasty in women (12.2%; 95% CI, 6%-19%), wasted legs (11%; 95% CI, 7%-14%), and sunken face (9%; 95% CI, 5%-12%). Figure 4 shows the Kaplan-Meier estimate of the probability of developing each type of these morphologic alterations during the follow-up period.

Table 2 reports the crude and adjusted RHs of developing any morphologic alterations from fitting a proportional hazards Cox regression model. Female sex and HCV status were independently associated with an increased risk of developing any morphologic alterations (RH, 4.01, and P<.001 compared with men; and RH, 2.43, and P=.01 compared with HCV-negative individuals). Also, there was evidence of older patients being at higher risk of developing any alterations (RH, 1.31 per 10 years older; P=.05). Patients who acquired human immunodeficiency virus infection via injection drug use were at lower risk of developing morphologic alterations compared with all other transmission routes. The duration of the treatment with any of the drugs administered was not associated with a significantly increased risk of developing ATA. Of note, duration of stavudine treatment was linked to an increased risk in univariate analysis (P=.03) but not in multivariate analysis (P=.13). Results were similar when this analysis was conducted separately on patients starting dual nucleoside therapy or HAART (data not shown). However, patients who started with 2 NRTIs and subsequently added a PI had a significantly higher risk of ATA compared with those who started with 2 NRTIs and did not add a PI during the period of observation (RH, 3.66; P=.007), while patients who started with HAART from a naive state showed a risk 2.42 times higher than that of patients who continued taking 2 NRTIs, although not significant (P=.10). Of the 103 patients who added a PI to their ART combination, 51 did it after virologic failure (viral load, >500 copies in 2 consecutive determinations). A concomitant switch from zidovudine to stavudine was done in 10 cases. The risk of ATA observed in patients who had virologic failure or who had replaced zidovudine with stavudine before adding the PI was not significantly increased compared with that of the other patients who added PIs.

In a multivariate Cox model, the increased risk of ATA associated with the addition of a PI to the dual nucleoside regimen remained statistically significant after adjusting for stavudine switching, previous viro-
logic failure, and CD4 cell variation after initiating ART (this latter as a time-dependent covariate; RH, 2.79; 95% CI, 1.28-6.1; \( P = .01 \)).

**Table 3** reports the adjusted risk of developing morphologic alterations separately for each type of ATA. Women were at higher risk than men for types 2 and 3, and non–intravenous drug users had a higher risk of developing both types 1 and type 2 ATA compared with subjects who become infected through intravenous drug use.

The HCV-positive status was significantly associated with an increased risk of developing lipoatrophy, independent of the other factors included in the analysis (RH, 4.74; \( P = .02 \)), but not the other types of ATA.

The duration of exposure to stavudine was associated with a significantly increased risk of developing lipoatrophy (RH, 23.56 per year longer of exposure; \( P = .04 \)); however, an increased, albeit not statistically significant, risk of developing type 1 ATA was correlated also with duration of zidovudine treatment (RH, 10.34; \( P = .12 \)). On the contrary, the combined form (type 3 ATA) seemed to be unrelated to the time spent taking both of these drugs and significantly associated with indinavir use (RH, 4.78 per year longer of exposure; \( P = .03 \)). Again, results were similar when the models were fitted separately in patients who started 2 NRTIs or HAART (data not shown).

Of note, among patients who started 2 NRTIs, the risk of having any ATA associated with the duration of dual nucleoside therapy tended to be higher in those who added a PI than in those who did not (RH, 1.86; 95% CI, 0.47-7.36; \( P = .38 \); and RH, 0.74; 95% CI, 0.25-2.21; \( P = .59 \), respectively). This difference was stronger, yet still not statistically significant, when the end point was fat wasting alone (RH, 3.43; 95% CI, 0.21-55.46; \( P = .38 \) vs RH, 0.69; 95% CI, 0.14-3.45; \( P = .65 \), respectively).

**COMMENT**

The present study is one of the largest performed until now to investigate the dynamics of ATAs in patients recently entered in their first-line ART. Moreover, it offered the opportunity to compare patients starting with PI-including therapies with patients starting with 2 NRTIs. Furthermore, the presence of a large proportion of women (27.9%) gave us the opportunity to assess sex-related differences in presentation and in the correlates of risk of developing different types of ATA.

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Table 2. Estimated Relative Hazards of Developing Any Morphologic Alterations From Fitting a Proportional Hazard Cox Regression Analysis*

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Crude RH (95% CI)</th>
<th>Crude P Value</th>
<th>Adjusted RH (95% CI)</th>
<th>Adjusted P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, 10 y older</td>
<td>1.30 (1.06-1.60)</td>
<td>.01</td>
<td>1.31 (1.00-1.72)</td>
<td>.05</td>
</tr>
<tr>
<td>Women</td>
<td>1.78 (1.22-2.61)</td>
<td>.003</td>
<td>4.01 (2.05-7.85)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HIV exposure†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual</td>
<td>2.07 (1.20-3.59)</td>
<td>.009</td>
<td>4.95 (1.92-12.80)</td>
<td>.001</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>1.88 (1.19-2.95)</td>
<td>.007</td>
<td>2.12 (0.93-4.83)</td>
<td>.07</td>
</tr>
<tr>
<td>Other</td>
<td>2.84 (1.41-5.73)</td>
<td>.004</td>
<td>6.09 (2.29-16.18)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CD4 count, per every 100-cells/µL</td>
<td>0.97 (0.89-1.05)</td>
<td>.42</td>
<td>0.94 (0.83-1.06)</td>
<td>.33</td>
</tr>
<tr>
<td>Viral load, log(10) copies/mL</td>
<td>1.00 (0.80-1.25)</td>
<td>.99</td>
<td>0.90 (0.66-1.23)</td>
<td>.51</td>
</tr>
<tr>
<td>HCV positive</td>
<td>0.91 (0.60-1.38)</td>
<td>.65</td>
<td>2.43 (1.19-4.95)</td>
<td>.01</td>
</tr>
<tr>
<td>Weight, 10 kg heavier</td>
<td>0.99 (0.86-1.14)</td>
<td>.90</td>
<td>1.18 (0.98-1.42)</td>
<td>.07</td>
</tr>
<tr>
<td>Time on treatment regimen (1 y longer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>1.03 (0.76-1.39)</td>
<td>.86</td>
<td>0.74 (0.21-2.65)</td>
<td>.64</td>
</tr>
<tr>
<td>Stavudine</td>
<td>1.44 (1.04-2.01)</td>
<td>.03</td>
<td>2.56 (0.75-8.74)</td>
<td>.13</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>0.85 (0.63-1.16)</td>
<td>.32</td>
<td>1.87 (0.57-6.15)</td>
<td>.30</td>
</tr>
<tr>
<td>Didanosine</td>
<td>0.93 (0.64-1.38)</td>
<td>.71</td>
<td>0.63 (0.18-2.33)</td>
<td>.47</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>1.40 (0.89-2.21)</td>
<td>.14</td>
<td>1.08 (0.30-3.90)</td>
<td>.91</td>
</tr>
<tr>
<td>Indinavir</td>
<td>1.01 (0.73-1.39)</td>
<td>.95</td>
<td>0.86 (0.42-1.77)</td>
<td>.68</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>1.36 (0.89-2.08)</td>
<td>.16</td>
<td>1.07 (0.50-2.30)</td>
<td>.86</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>1.37 (0.51-3.70)</td>
<td>.54</td>
<td>0.85 (0.20-3.74)</td>
<td>.83</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>1.07 (0.58-1.98)</td>
<td>.84</td>
<td>1.00 (0.41-2.44)</td>
<td>.99</td>
</tr>
<tr>
<td>2 NRTIs</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2 NRTIs + PI (first line)</td>
<td>1.50 (0.85-2.64)</td>
<td>.16</td>
<td>2.42 (0.85-6.88)</td>
<td>.10</td>
</tr>
<tr>
<td>2 NRTIs + PI (deferred)</td>
<td>2.11 (1.03-4.24)</td>
<td>.04</td>
<td>3.66 (1.43-9.34)</td>
<td>.007</td>
</tr>
</tbody>
</table>

* RH indicates relative hazard; CI, confidence interval; HIV, human immunodeficiency virus; HCV, hepatitis C virus; NRTIs, nucleoside reverse transcriptase inhibitors; and PI, protease inhibitor.
† Versus intravenous drug user.
received a potent PI in the first-line treatment, the observed incidence of ATA confirms the high risk of occurrence of these alterations, even irrespective of the drug combination used. Fat loss was observed in 150 body sites of 72 patients and fat accumulation in 114 sites of 85 patients. Of note, the concomitant presence of loss and accumulation in different body sites was reported in only 29 cases (4.4%).

In other studies, “mixed lipodystrophy,” otherwise defined as combined form, was more frequent than in our study population. In particular, in the study by Martinez et al,12 who included only patients starting PI-including treatments, the percentage of patients with combined forms was 7.7% during a median follow-up of 18 months.

Our study also showed that the time to the onset of ATA is significantly different according to the different body sites involved. Abdominal fat accumulation was seen earlier than the other ATAs, while sunken face required longer to appear, suggesting that fat loss may be a consequence of long exposure to ART.

Overweight before ART has been reported to be a predictive factor of further fat accumulation during ART.17 The frequency and the timing of presentation of abdominal fat accumulation in our cases may be explained by the pre-ART body mass index of our patients, which was in the normal range, or slightly higher than in the uninfected Italian population of the same age.

In our study population, to have been HIV-infected by injecting drug use was a correlate of protection against ATA development. Similar findings have been reported by Martinez et al.12 The most obvious explanation, by looking at the data on adherence to ART of intravenous drug users, could be that this group has been less adherent to therapy. However, only 44 patients were active drug users at the time of our study (6.7% of the total and 18.6% of the subjects classified as intravenous drug users). This finding suggests that behavioral or social factors other than active drug use, such as low level of education and belonging to a low-income class, may have played a role in reducing adherence to ART and consequently increasing the risk of ATAs.16

On the contrary, the RH of developing ATAs in patients who started ART with PI-including triple combinations was not significantly higher when compared with the risk in patients who received 2 NRTIs as their first ART regimen. In contrast, patients who added a PI to a dual-NRTI regimen during the follow-up had a risk of developing ATAs that was significantly higher (about 2 times greater) than the hazard in patients continuing to take a 2-NRTI combination. Interestingly, this increased risk was significant, independent of previous virologic failure, switch from zidovudine to stavudine, or CD4 cell variations during ART. Moreover, the risk of ATA associated with the time of exposure to NRTIs tended to be higher in the group of patients who added a PI during the follow-up. These findings suggest that these 2 classes of antiretroviral drugs cooperate in inducing ATAs, and that previous exposure to NRTIs may exacerbate the risk of ATAs associated with the exposure to PIs, and support the hypothesis that biological modifications induced by the treatment as a whole (ie, modification of cytokine production or rescue and/or suppression of specific immune functions persisting for a long time in chronically infected patients) and not only specific drug toxicity are involved in causing ATAs.
The possible existence of different pathogenic mechanisms involved in ATAs may be supported by the results of the analysis on the correlates of risk of each ATA type. Types 2 and 3, but not type 1 (lipodystrophy), are significantly more frequent in women. Lipodystrophy increased significantly per every year spent taking stavudine, independent of sex. Of interest, even the duration of treatment with zidovudine, the alternative drug to stavudine, is associated with an increased risk of lipodystrophy, albeit not statistically significant. In both cases, the large CIs observed oblige us to be cautious in drawing definitive conclusions on the role of each of these drugs in causing ATAs. However, these findings further support a role of NRTIs in the etiology of lipodystrophy. In fact, duration of exposure to PI did not increase significantly the risk of developing ATAs. The strong relationship between female sex and developing 2 NRTIs significantly increases the risk of developing these abnormalities. Similar findings were reported by Martinez et al.12 Taken together, these findings suggest that lipodystrophy is largely independent of sex and probably related to NRTI treatment.

Interestingly, HCV coinfection, highly prevalent in our cohort, was independently linked to an increased risk of developing lipodystrophy. This finding is in agreement with recent observations reported by Duong et al13 in a small HAART-treated group of patients with high prevalence of lipodystrophy. The HCV-induced liver damage may contribute to abnormal insulin sensitivity, whose main effect would be adipogenesis inhibition accompanied by lipolysis stimulation.19 A second mechanism by which HCV could increase drug toxicity is a reduced drug metabolism due to hepatic cell damage that might result in cumulative adverse effects over time. In the absence of specific data, studies addressing this issue are needed to better clarify the role of HCV coinfection in influencing and predicting lipodystrophy in patients receiving antiretroviral drugs.

Type 2 ATA (fat accumulation) seemed not to be related to any specific drug exposure. The fact that the major risk, albeit not significant, has been observed in association with duration of exposure to both stavudine and zidovudine (drugs that are alternatively present in most of the ART combinations prescribed to our patients) suggests that this form may be simply linked to the duration of ART. Nevertheless, specific hormonal mechanisms in women cannot be excluded.

On the contrary, there was evidence of a role of potent PI in combined forms (type 3 ATA). In fact, patients exposed to indinavir, the PI most frequently used and for the longest time in our study, showed a significantly increased risk of developing these abnormalities.

This investigation is mainly based on clinical observation and may have significant limitations because of the subjective physician assessment of the observed phenomena. To overcome these potential biases, the same physician was requested to give a judgment in all the follow-up visits in each center, using a standardized form chart.

A second caveat could be the partially retrospective study design, chosen to have the possibility of obtaining information in a short time. However, all the centers participating in IcoNa were alerted to the occurrence of ATAs about 1 year before the time of LipoICoNa design and enrollment. Thus, most study data were actually collected prospectively. Dietary changes could be another confounding factor able to modify adipose tissue distribution independently from drugs. Moreover, ART initiation in patients with advanced disease is often associated with an increased body weight.20 However, in only less than 2% of the enrolled patients have significant dietary changes been traced on follow-up sheets before the appearance of metabolic or morphologic alterations, and they seemed to be irrelevant to the development of ATAs in these patients.

In conclusion, ATA incidence is already high in patients starting first-line ART after around 2 years of therapy. Risk is conditioned by several variables, including behavioral and social factors probably altering the adherence to the therapy. Metabolic alterations derived from the hepatic damage caused by HCV infection could be involved. Use of NRTIs (particularly stavudine) seems to be related to lipodystrophy development, and the addition of a PI in the regimen in patients previously receiving 2 NRTIs significantly increases the risk of developing ATA. The strong relationship between female sex and types 2 and 3 ATAs remains to be explained on a biological basis.

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REFERENCES


